COUNTWAY LIBRARY
HC 6DW6

BOSTON MEDICAL LIBRARY
IN THE
FRANCIS A. COUNTWAY
LIBRARY OF MEDICINE

THE ANATOMICAL RECORD

19728

EDITORIAL BOARD

IRVING HARDESTY
Tulane University

CLARENCE M. JACKSON
University of Minnesota

THOMAS G. LEE
University of Minnesota

FREDERIC T. LEWIS
Harvard University

WARREN H. LEWIS
Johns Hopkins University

CHARLES F. W. MCCLURE
Princeton University

WILLIAM S. MILLER
University of Wisconsin

FLORENCE R. SABIN
Johns Hopkins University

GEORGE L. STREETER
University of Michigan

G. CARL HUBER, Managing Editor
1330 Hill Street, Ann Arbor, Michigan

VOLUME 20

DECEMBER, 1920—MARCH, 1921

PHILADELPHIA
THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY

BOSTON MEDICAL
MAY 9 1921
LIBRARY

CATALOGUED
MAY 9 1921
M. E. C.

CONTENTS

NO. 1. DECEMBER, 1920

GEORGE S. HUNTINGTON AND CHARLES F. W. MCCLURE. The development of the veins in the domestic cat (Felis domestica), with especial reference, 1) to the share taken by the supracardinal veins in the development of the postcava and azygos veins and, 2) to the interpretation of the variant conditions of the postcava and its tributaries, as found in the adult. Twelve figures (in colors) 1

E. V. COWDRY. Anatomy in China. One illustration 31

EUGENE L. SETTLES. The effect of high fat diet upon the growth of lymphoid tissue. Sixteen figures (three plates) and one chart 61

IVAN E. WALLIN. A case of persistent left supracardinal vein with two left spermatic veins. One figure 95

NO. 2. JANUARY, 1921

E. T. HSIEH. A review of ancient Chinese anatomy. Thirty-one figures 97

OLIVER H. GAEBLER. Bladder epithelium in contraction and distention. Nine figures. 129

WALTER N. HESS. Tracheation of the light-organs of some common Lampyridae. Ten figures 155

Proceedings of the American Society of Zoologists. Eighteenth Annual Meeting 163

Proceedings of the American Society of Zoologists. Abstracts 181

Proceedings of the American Society of Zoologists. Constitution, etc. 233

Proceedings of the American Society of Zoologists. List of Officers and Members. ... 237

NO. 3. FEBRUARY

JUAN C. NAÑAGAS. Two cases of monoventricular heart with atresia and transposition of some of the roots of the great vessels. Eight figures (two plates) 255

H. E. JORDAN. Further evidence concerning the function of osteoclasts. Five figures. 281

PAUL K. WEBB AND JAMES BARRETT BROWN. A case of independent costal bars of the epistropheus in man. Two figures 297

VINCENT VERMOOTEN. A study of the fracture of the epistropheus due to hanging with a note on the possible causes of death. Twelve figures 305

P. E. LINEBACK. A case of unilateral polydactyly in a 22-mm. embryo. One figure 313

NO. 4. MARCH

HELEN DEAN KING. A comparative study of the birth mortality in the albino rat and in man 321
DAVID M. SIPERSTEIN. The effects of acute and chronic inanition upon the development and structure of the testis in the albino rat. Five plates (fourteen figures).... 355
BRADLEY M. PATTEN AND REES PHILPOTT. The shrinkage of embryos in the processes preparatory to sectioning. Eight figures..... 393
CHARLES H. MILLER. Demonstration of the cartilaginous skeleton in mammalian fetuses 415
ALEXANDER GIBSON. Note on a persistent left duct of Cuvier. One figure..... 421

AUTHORS' ABSTRACT OF THIS PAPER ISSUED BY THE BIBLIOGRAPHIC SERVICE, NOVEMBER 15

BOSTON MEDICAL
MAY 9 1921
LIBRARY

THE DEVELOPMENT OF THE VEINS IN THE DOMESTIC CAT (FELIS DOMESTICA) WITH ESPECIAL REFERENCE, 1) TO THE SHARE TAKEN BY THE SUPRACARDINAL VEINS IN THE DEVELOPMENT OF THE POSTCAVA AND AZYGOS VEINS AND 2) TO THE INTERPRETATION OF THE VARIANT CONDITIONS OF THE POSTCAVA AND ITS TRIBUTARIES, AS FOUND IN THE ADULT

GEORGE S. HUNTINGTON
Columbia University

AND

CHARLES F. W. McCLURE
Princeton University

TWELVE FIGURES (IN COLORS)

This investigation was begun in 1905. In the preceding years we had found that the variations or atypical conditions of the venous system observed by Darrach,[1] McClure[2] and others in the adult cat could not all be explained on the basis of the existing knowledge of the development of the veins. We therefore determined to study in detail the normal ontogenetic plan of the veins in the cat, with the hope that we might be able to interpret correctly the variant conditions of the venous system which arose as the result of atypical development of the veins.[3]

We have recently extended our observations to a study of the development of the veins in man, the pig and the rat, and while

[1] Darrach, W. 1907. Variations in the postcava and its tributaries as observed in 605 examples of the domestic cat. Proc. Amer. Ass. Anat., Amer. Jour. Anat., Vol. 6.

[2] McClure, C. F. W. 1900. On the frequency of abnormalities in connection with the postcaval vein and its tributaries in the domestic cat (Felis domestica). Amer. Nat., Vol. 34.

[3] Huntington, G. S., and McClure, C. F. W. 1907 The Development of the Postcava and Tributaries in the Domestic Cat. Proc. Amer. Ass. Anat., Amer. Jour. Anat., Vol. 6.

it has been observed that important generic differences exist in the pig and the rat, the development of the veins in man has been found to resemble more closely that observed in the cat.[4]

Although our investigation has been completed, it may be some time before the detailed publication is ready for the press. We have therefore deemed it advisable to publish a preliminary summary of our work in the form of a series of diagrams which illustrate the normal ontogenetic plan followed by the veins, with especial reference, however, to the share taken by the supracardinal system of veins in the development of the azygos vein and of the postrenal division of the postcava in the cat. A brief summary is also given of the potential postcaval variants in the adult cat and man. An explanation of these variant conditions is based on a composite diagram (fig. 12) combining all of the venous pathways which may arise during ontogeny and which are potentially capable of being retained in the adult in atypical individuals.

The diagrams which we present are based on a large series of wax reconstructions made after the method of Born, of cat embryos ranging between 5 and 45 millimeters in length. They represent a series of critical ontogenetic stages leading from the early primitive venous groundplan common to all vertebrates (fig. 1), up to the conditions obtaining in the adult cat.

In constructing these diagrams it was deemed desirable to include all of the main axial venous channels of the body other than those investigated by us, and we fully appreciate and acknowledge that the previous contributions of other investigators, especially those of Rathke,[5] Hochstetter[6] and F. T. Lewis,[7] have made this possible.

[4] Huntington, G. S. and McClure. C. F. W. 1920. A series of diagrams explanatory of the development of the postcava in the cat, with especial reference to the share taken by the supracardinal system of veins. Proc. Amer. Ass. Anat., Anat. Rec., Vol. 18.

[5] Rathke, H. 1838. Ueber den Bau und Entwicklung des Venensystems der Wirbeltiere. Dritte Bericht über das Naturwissenschaftliche Seminar zu Königsberg. Idem. 1832. Abhandlungen zur Bildungs-und Entwicklungsgeschichte des Menschen und der Thiere. Leipzig, T. I.

[6] Hochstetter, F. 1893 Beiträge zur Entwickelungsgeschichte des Amnioten, III, Säuger, Morph. Jahrb., Bd. 20.

[7] Lewis, F. T. 1902 The development of the vena cava inferior. Amer. Jour. Anat., Vol. 1.

The uniform color scheme shown in the figures makes it easy to follow the transformations of the embryonic venous system through the different stages of its development. Only a brief account of each critical stage will be given in the present paper, while a more detailed description will be reserved for a later publication in which the actual reconstructions will be demonstrated on which our observations have been based.

FIG. 1. CARDINO-SUBCARDINAL STAGE.

This stage of development may be regarded as representing a common groundplan of the vertebrate embryonic venous system. It forms the starting point which is succeeded by a series of modifications gradually leading up to the conditions observed in the adult. In some cases these modifications are brought about by the persistence or atrophy of certain of its component vessels, while in others, modifications arise by the addition of newly-formed vessels to the primary groundplan. The diagrams we present illustrate these modifications of the embryonic groundplan as observed in the embryo of the cat.

The principal vessels of the embryonic venous groundplan consist of three pairs of essentially bilateral symmetrical veins, viz.: the precardinal (*Prc.*) and postcardinal (*Pc.*) veins which open in common into the sinus venosus through the ducts of Cuvier (*D.C.*) and the subcardinal (*Subc.*) veins. The postcardinal veins lie dorsal to the mesonephroi, while the subcardinals lie ventro-medial to the same. Anastomoses between the postcardinal and subcardinal veins are present and intersubcardinal anastomoses are met with caudal to the origin from the aorta of the omphalomesenteric artery.

The sex veins from the gonads (*G*) open into the subcardinal veins at this stage. The veins of the liver drain for the most part directly into the sinus venosus through the *V.* hepatica communis (*V.H.C.*) and no connection between the liver circulation and the right subcardinal vein has as yet been formed.

The blood collected from the body-walls and mesonephroi by the postcardinals, as well as that carried by the subcardinal veins, is returned to the heart through the ducts of Cuvier (*D.C.*) in common with that brought from the head region and anterior limb-buds by the precardinal veins.

FIG. 2. ESTABLISHMENT OF THE PARS HEPATICA AND PARS SUBCARDINALIS OF THE POSTCAVA, CONSTITUTING THE PRERENAL DIVISION OF THE POSTCAVA IN THE ADULT.

The significant features of this stage of development, are the establishment of the prerenal division of the adult postcava, and the beginning replacement of an originally bilaterally symmetrical by a subsequent asymmetrical plan of axial venous organization.

As shown by F. T. Lewis, the prerenal division of the postcava is formed by the establishment of a communication between the veins of the liver and the right subcardinal vein. The point at which this communication is established with the right subcardinal has been previously designated by one of the writers[8] as the hepato-subcardinal junction (*Hep.Subc.Jct.*).

The portion of the prerenal division of the postcava which lies cranial to the hepato-subcardinal junction may be termed the *pars hepatica* (*P.Hep.*). It is formed *in situ* by a rearrangement and transformation of vascular channels already formed, viz., by the *V.* hepatica communis, the hepatic sinusoids and by the vascular area formed within the caval mesentery.

The portion of the prerenal division of the postcava which lies caudal to the hepato-subcardinal junction is known as the *pars subcardinalis* (*P.Subc.*), as it is formed largely by a portion of the right subcardinal vein. Slightly caudal to the origin of the omphalomesenteric artery from the aorta, the pars subcardinalis establishes a wide communication with the left subcardinal, through an intersubcardinal anastomosis (*Int.Subc.Anast.*) and also one with each postcardinal, by the enlargement of one of the anastomoses that exist at this level between the intersubcardinal anastomosis and the postcardinal veins (subcardino-postcardinal anastomoses, *Subc.Pc.Anast.* (fig. 12)).

As the result of these modifications of the early embryonic groundplan, the blood from the region of the body which lies caudal to the intersubcardinal anastomosis (body-walls and mesonephroi) is now, for the most part, directed from the postcardinal (*Pc.2*), on both sides of the body, through the subcardino-post-

[8] McClure, C. F. W. 1906 A contribution to the anatomy and development of the venous system of Didelphys marsupials (L.). Amer. Jour. Anat., Vol. 5, p. 171.

cardinal anastomosis to the prerenal division of the postcava. Also, correlated with these changes, the postcardinals (*Pc.2*), caudal to the intersubcardinal anastomosis, become greatly enlarged, while the segments cranial (*Pc. 1*) to this anastomosis become reduced and return blood from the body-walls of the thoracic region and from the cranial end of the mesonephroi directly to the heart by the ducts of Cuvier (*D.C.*).

The sex veins still open into the subcardinal veins. Anastomoses are also still present between the subcardinal and postcardinal veins, caudal to the intersubcardinal anastomosis, which form part of the mesonephroic circulation. In the region cranial to the intersubcardinal anastomosis, however, the subcardinals soon become associated with the anlagen of the adrenal bodies forming the main venous drainage line of these organs which is retained throughout subsequent stages of development.

FIG. 3. FURTHER TRANSFORMATIONS OF THE POSTCARDINAL AND SUBCARDINAL VEINS WHICH ARE ASSOCIATED LARGELY WITH THE GROWTH OF THE MESONEPHROS.

SEPARATION OF THE POSTCARDINAL VEINS INTO A THORACIC AND LUMBAR DIVISION.

The originally continuous postcardinals have now become divided into a cranial or thoracic (*Pc.1*), and into a caudal or lumbar (*Pc.2*) pair of veins. The former collect the blood chiefly from the body-walls of the thorax and from the anterior portion of the mesonephroi, while the latter drain the more caudal regions of the body.

The lumbar division of the postcardinal (*Pc.2*), on each side of the body, now encircles the relatively large mesonephros, both dorsally and medially, so that in reconstructions of the veins at this stage each mesonephros appears to be arched over by or to lie within a great venous basket or trough formed by the postcardinal vein and its subcardinal anastomoses.

The permanent kidneys (*K*) have migrated craniad from their earlier position ventral to the umbilical arteries and now occupy a position dorsal to the lumbar postcardinals (*Pc.2*).

Cranial to the intersubcardinal anastomosis (*Int.Subc.Anast.*) the subcardinal veins, except that portion of the vein of the right

side which forms the pars subcardinalis of the postcava (*P.Subc.*), have become still more closely associated with the adrenal organs so that from now on, we may speak of these veins as the adrenal veins (*Adr.*). Caudal to the intersubcardinal anastomosis the subcardinal veins have lost their original continuity so that the sex veins, of subcardinal origin, now drain directly into the postcardinals (*Pc.2*), as well as into the intersubcardinal anastomosis. The significance of this observation lies in the circumstance that the sex veins at this time are exclusively of subcardinal origin, a condition which is retained in the adult of lower vertebrates, up to and including birds.

FIG. 4. THE ESTABLISHMENT OF THE SUPRACARDINAL SYSTEM OF VEINS AND THE RENAL COLLAR.

A bilateral and originally symmetrical venous channel develops dorso-medial to the primitive postcardinal and dorso-lateral to the aorta, into which the somatic postcardinal tributaries secondarily drain. This secondary venous channel forms what we have termed the *Supracardinal System of Veins* (*Sprc.*). It extends, from the level at which the posterior limb veins unite with the postcardinals, to a point craniad where it joins that portion of the postcardinals (*Pc.1*) which alone persists to form the cranial end of the adult azygos (*Az.*) veins. Between these levels the supracardinal veins come to enter into the definite organization of both the adult postcava in its postrenal division and of the azygos in its lumbar and part of its thoracic segments, entirely replacing in these districts the primitive postcardinal veins.

"It is important to note that the supracardinal veins are not in any sense merely synonyms for the dorsal limb of the periureteric ring described by Hochstetter and others, but comprise a continuous morphologically uniform system of longitudinal venous channels which contribute to the establishment of the adult condition in both the postcaval and azygos areas."[9]

The beginning of the establishment of the supracardinal system of veins takes place at a relatively early stage of development, and its cranial extension into the thoracic region is evidence that its appearance is not primarily associated with the cranial migration of the permanent kidneys. Frequent anastomoses occur

[9] Loc. cit., footnote 3.

between the postcardinals and supracardinals at an early stage of development and, at this time, the anastomoses between the right and left supracardinal veins (intersupracardinal anastomoses), as shown in figure 4, have not yet been formed.

The early anastomoses between the supracardinal and postcardinal veins soon undergo atrophy and disappear. A large single anastomosis (fig. 4, *R.Col.*), at about the level of the intersubcardinal anastomosis, is retained, however, on each side of the body, which permits blood collected by the supracardinals in the lumbar region to reach the heart by way of the prerenal division of the postcava. This anastomosis we have designated as the subcardino-supracardinal anastomosis (*R.Col.*, fig. 4), since, in the later stages, it appears to connect the subcardino-postcardinal anastomosis with the supracardinal vein.

The blood collected by the supracardinal veins which is not directed to the prerenal segment of the postcava through the subcardino-supracardinal anastomosis, reaches the heart by way of the ducts of Cuvier (*D.C.*).

The formation of this subcardino-supracardinal anastomosis, at about the level of the intersubcardinal anastomosis, establishes the presence of a circum-aortic venous ring at this point which we have designated as the *Renal Collar.*

In order to appreciate the topographical relations of the renal collar to the main venous channels and the aorta, the reader is referred to fig. 12, a diagram based largely on the reconstruction of a 16 millimeter embryo of the cat. As clearly shown here, the renal collar is formed by the pars subcardinalis of the postcava (*P.Subc.*), the intersubcardinal anastomosis (*Int.Subc. Anast.*), the right and left subcardino-postcardinal anastomoses (*Subc.Pc.-Anast.*), the right and left subcardino-supracardinal anastomoses (*Subc.Sprc.Anast.*), the right and left supracardinals (*B* and *C*) and the anastomoses between the supracardinals dorsal to the aorta at this point, at which level the renal veins (*R.V.*) enter the collar.

The sex veins (fig. 4) still join the postcardinals and, in some cases, as shown in fig. 3, they may also open into the intersubcardinal anastomosis, a condition which is occasionally retained in the adult when multiple sex veins are present.

FIGURE 5. PROGRESSIVE DEVELOPMENT OF THE BILATERAL, SYMMETRICAL SYSTEM OF SUPRACARDINAL VEINS.

SEPARATION OF THE SUPRACARDINALS INTO AN AZYGOS AND LUMBAR DIVISION.

COMPLETION OF THE CRANIAL MIGRATION OF THE PERMANENT KIDNEYS, THE DEVELOPMENT OF THE RENAL VEINS AND THE ESTABLISHMENT OF THE PERIURETERIC VENOUS RINGS.

The supracardinal veins (*Sprc.*) at first undergo a progressive development during which their bilateral symmetry is retained for a considerable period of time. They soon separate, however, into a cranial or azygos and into a caudal or lumbar pair of veins, from which the azygos veins and a segment of the postrenal division of the adult postcava are, respectively, derived.

The bilateral symmetrical pair of supracardinals in the lumbar region anastomose freely with each other dorsal to the aorta and become greatly increased in size. Blood which they receive from the body-walls through four pairs of dorsal tributaries, and that received by them from the external (*E.Il.*) and internal iliac (*I.Il*) veins, now reaches the prerenal segment of the postcava exclusively through the right and left subcardino-supracardinal anastomoses (lateral portion of renal collar, *R.Col.* fig. 5), which have become correspondingly enlarged.

Blood collected by the supracardinals in the thoracic region (azygos veins), chiefly from the body-walls, is returned to the heart through the right and left ducts of Cuvier (*D.C.*), in common with that collected from the head region and anterior limbs by the precardinal veins.

The permanent kidneys (*K*) have completed their migration craniad, and the hilus of each kidney now lies at about the level of the renal collar (fig. 12). After the migration of the kidneys has been completed, the permanent renal veins (*R.V.*) are formed. These consist at first of a right and left pair of renal veins which extend between the hilus of each kidney and the lateral portion of the renal collar (*R.V.*, fig. 12).

The relation of the renal veins to the lateral portion of the renal collar (subcardino-supracardinal anastomosis) is shown in figs. 8 and 9 which are lateral views of the right side of actual recon-

structions of the veins of cat embryos measuring, respectively, 16 and 25 mm. in length. Near the hilus of the kidney, each renal vein divides into two branches (fig. 5) and, in some cases, the bifurcation may even extend back to the renal collar.

The primitive postcardinal veins (*Pc.2*, fig. 5) still retain their bilateral symmetry. They unite caudally with the supracardinals in the lumbar region and, in addition to blood received from the mesonephroi and gonads, they also receive, in part, that collected by the external (*E.Il.*) and internal iliac (*I.Il.*) veins. Craniad, the postcardinals (*Pc.2*) also unite with the supracardinals (*Sprc.*) through the subcardino-supracardinal anastomosis (lateral portion of renal collar, *R.Col.*, fig. 5) so that the blood collected by the supracardinals and postcardinals in the lumbar region is then conveyed to the prerenal division of the postcava, on each side of the body, through the original subcardino-postcardinal anastomoses (*Subc.Pc.Anast.*, fig. 12) which constitute the ventral portions of the renal collar. (Compare figs. 5 and 12.)

As the result of this cranial and caudal union in the lumbar region between the postcardinal and supracardinal veins, a venous ring has been established on each side of the body, through which the ureter (*Ur.*) passes. This periureteric venous ring is bounded cranially by the caudal border of the subcardino-supracardinal anastomosis (*R.Col.*, figs. 5 and 8 and *Subc.Sprc.Anast.*, fig. 12), dorsally by the supracardinal and ventrally by the postcardinal vein. In the region of the renal collar the ureter (*Ur.*) lies dorsal to the postcardinal (*Pc.2*) and sex vein, while further caudad it passes ventral to the postcardinal vein.

Final Transformations of the Veins (Figure 6) Which Lead Up to the Conditions Found in the Adult Cat (Figure 7).

The primary bilaterally symmetrical plan of the supracardinal veins in the lumbar region (fig. 5, *Sprc.*) is soon replaced by an asymmetrical one (fig. 6). This change is initiated by a reduction of the left side of the circum-aortic venous ring, involving its supracardinal component and of the portion labelled *R.Col.*, fig. 6, while the corresponding channels of the right side enlarge. This

change is finally followed by the complete atrophy of the left side of the renal collar, or strictly speaking, of the subcardino-supracardinal anastomosis of the left side.

In consequence of this change, the blood collected by the left supracardinal vein is therefore directed toward the right supracardinal through the intersupracardinal anastomoses and, together with the blood collected by the right supracardinal, now reaches the prerenal division of the postcava by way of the right side of the renal collar. Fig. 6 represents a stage in which the left side of the renal collar is undergoing atrophy, while in fig. 7, the left side of the collar has completely disappeared.

While it may be said that the right supracardinal vein is chiefly concerned in the formation of a portion of the postrenal division of the postcava, the supracardinal vein of the left side is also involved. The left supracardinal (fig. 6) does not, as one might suppose, undergo complete atrophy *in situ*, but rather is drawn into or fuses with the vein of the right side, at least in the cadual half of its extent. This portion of the postcava derived from the supracardinals (pars supracardinalis, fig. 7) may therefore be regarded as being formed largely through a fusion of both supracardinal veins, and not from a single vein on the right side, as is usually described to be the case, although the latter furnishes the major contribution.

It has been stated above that after the complete atrophy of the left side of the renal collar, all of the blood collected by the supracardinals in the lumbar region reaches the prerenal division of the postcava through the right side of the renal collar. One of the most interesting observations we may have made regarding the development of the veins in the cat, is that the right side of the renal collar typically enters directly into the formation of a definite portion of the postrenal division of the postcava, which we have designated the *pars renalis* (*P.Ren.*) of the postcava, on account of its relation to the entrance of the renal veins (*R.V.*, fig. 7). The manner in which this is brought about can best be illustrated by a series of actual reconstructions of the veins which show the relations that the right side of the renal collar bears to

the prerenal division of the postcava and to the supracardinal and postcardinal veins. The reconstructions referred to are represented by figs. 8, 9, 10 and 11 which are lateral views of the veins of the right side of cat embryos measuring 16, 25, 29 and 45 mm., respectively, in length.

In the 16 mm. embryo (fig. 8) it is seen that the right periureteric venous ring is still complete and that blood from the caudal region of the body can reach the prerenal division of the postcava by two routes, viz., by way of the right postcardinal vein (*Pc.2*), into which the sex veins open, and by way of the right supracardinal vein and right side of the renal collar. This latter route is the permanent one typically retained in the adult and *constitutes the postrenal division of the postcava.* In the 16 mm. embryo, from which this figure was drawn, the left side of the renal collar is also intact, so that blood from the left side of the body can also reach the prerenal segment of the postcava through a corresponding set of veins (fig. 5).

In the 25 mm. embryo (fig. 9) both postcardinal veins (*Pc.2*) have given up their caudal connection with the external and internal iliac veins and the left side of the renal collar has also completely disappeared, so that blood from the hind limbs and lumbar region of the body, now reaches the prerenal division of the postcava solely through the fused supracardinals and the right side of the renal collar. The right postcardinal vein opens into the ventro-caudal border of the renal collar (figs. 9 and 12) and returns blood to the prerenal segment of the postcava, which it receives exclusively from the right mesonephros and from the right gonad through the sex vein.

The venous arch formed by the fused supracardinals and right side of the renal collar, in figs. 8 and 9, is a marked and constant character of these veins in the earlier stages, a condition, however, which is later changed. This change consists of a straightening out of the arch formed by the supracardinals, and of an *actual elongation, in a cranio-caudal direction, of the right side of the renal collar.* Fig. 10, of a 29 mm. embryo, shows the renal collar in the process of elongation, while in fig. 11, of a 45 mm. embryo, the elongation has been completed and the collar assumed the

condition permanently retained in the adult. As a result of this elongation, the point at which the right postcardinal (*Pc.2*) opens into the renal collar becomes gradually shifted caudad, so that the right postcardinal finally opens into the postcava (renal collar) somewhat caudal to the points of origin of the right renal veins (*R.V.*, fig. 11).

The blood from the right gonad reaches the right postcardinal through the sex vein of subcardinal derivation. After the complete degeneration of the right mesonephros, the original drainage line of the right postcardinal is occupied exclusively by that of the right sex vein (figs. 8, 9,10, 11 and 7). The original point of connection of the right postcardinal with the renal collar, although shifted caudad, is therefore retained in the adult as the point at which the postcava is joined by the right sex vein. This point, as we have seen, is the ventro-caudal border of the right embryonic renal collar. In figs. 6 and 7, the elongation of the right side of the renal collar is represented as having taken place.

Considerable variation has been found to exist in the adult cat, as regards the relation of the points at which the right sex and the right renal veins open into the postcava. This variation can undoubtedly be explained, in some cases, at least, on the ground that the extent to which the right side of the renal collar elongates in a cranio-caudal direction may differ in individual cases. The opening of the right sex vein into the postcava, caudal to that of the right renal vein is, however, now easily explained, on the basis of an elongation of the right side of the renal collar. Furthermore, it is plainly evident that the *right postcardinal vein does not, in the slightest degree, as hitherto supposed, enter into the formation of the postrenal division of the postcava which, as we have seen, is formed by the supracardinal veins* (*P. Sprc.*) *and by the right side of the renal collar* (*P. Ren.*, fig. 7).

As far as the postcava and its tributaries are concerned, there still remain to be considered further changes regarding the right and left renal veins and the sex vein of the left side.

It has been stated above that after the migration of the kidneys has been completed, the permanent renal veins are formed. These consist at first of a right and left pair of renal veins which

extend between the hilus of each kidney and the lateral portion of the renal collar (*Subc. Sprc. Anast.*, fig. 12).

The condition which obtains on the right side of the body in the adult, appears to be brought about by the persistance of the more ventral of the two embryonic renal veins and the complete atrophy of the other (fig. 7). This single right renal vein then retains its connection with the pars renalis of the postcava which is formed from the right side of the renal collar (fig. 7).

The relations of the postcardinal vein, into which the sex vein opens, and of the two embryonic renal veins to the renal collar are originally the same on both sides of the body (figs. 5 and 12). When the left side of the renal collar (subcardino-supracardinal anastomosis) atrophies, however, the more ventral of the two embryonic renal veins and the left postcardinal vein continue to retain their connection with the left subcardino-postcardinal anastomosis. The result is that the latter, together with the more ventral of the two embryonic renal veins, then forms the left renal vein of the adult, while the left postcardinal vein, after the complete degeneration of the mesonephros, serves as the pathway through which the left sex vein opens into the left renal vein (compare figs. 6 and 7).

On the basis of their development, the presence in the adult cat of two, three or even four renal veins on the same side of the body is now not difficult to explain. When more than two renal veins are present this condition is undoubtedly related to the extent to which the bifurcation of the original embryonic renal veins is involved.

It has long been erroneously stated in text-books of embryology that the postcardinal veins in the thoracic region give rise to the azygos veins. The history of the development of the azygos veins in the cat is illustrated in figures 3 to 7, inclusive, in which it is shown that they are derived chiefly from the supracardinals and that in the adult cat only the proximal end of the right azygos near the duct of Cuvier, is derived from the postcardinal vein (*Pc.1*).

As stated at the beginning of this paper the chief object of our investigation was to establish the normal ontogenetic plan

of the veins in the cat, with the hope that we might be able to interpret correctly the variant conditions of the venous system which arose as the result of atypical development of the veins.[10] On the basis of this investigation we have therefore constructed a composite diagram (fig. 12) of the embryonic veins of the cat which make their appearance, at one time or another, during the course of ontogeny.

We have already shown in the preceding pages how some of these embryonic veins function only temporarily and then disappear, while others persist and are carried into the adult stage. When we meet with a venous variant in the adult it is therefore due, in the majority of cases, to the occurrence and persistence of some modification of the ontogenetic plan ordinarily followed by the veins, which is carried into the adult. It is also evident when such atypical conditions arise, that their explanation lies in the determination of the normal potential embryonic pathways which may have been concerned. Also, if our composite diagram of the embryonic veins is correct, we should not only be able to interpret the atypical conditions already found, but should also be able to predict those which are potentially capable of occurring in the adult cat.

On the basis of this composite ontogenetic plan of the veins we have been able to classify under 17 main *Types* the variant conditions of the postcava which may occur in the adult cat. We have also found that all of the postcaval variations thus far observed in man by other investigators, as well as by ourselves, can be classed under these same types.

If we return to the composite diagram (fig. 12) we see that the embryonic veins which typically enter into the formation of the adult postcava are the right supracardinal (*B*), the right subcardino-supracardinal anastomosis (*Subc.Sprc.Anast.*), the right subcardino-postcardinal anastomosis (*Subc.Pc.Anast.*), the intersubcardinal anastomosis (*Int.Subc.Anast.*), the pars subcardinalis (*P.Subc.*) and the pars hepatica (*P.Hep.*) of the postcava.

[10] Huntington, G. S. and McClure, C. F. W. 1907 The interpretation of variations of the postcava and tributaries of the adult cat, based on their development. Proc. Amer. Ass. Anat., Amer. Jour. Anat., Vol. 6.

On the other hand, it is plain that the persistence in the adult of the left supracardinal (*C*), or of the right postcardinal (*A*), or left postcardinal (*D*), either singly, in combination with one another, or with the right supracardinal vein (*B*) to form the postrenal division of the postcava, would constitute an atypical condition of the veins. In addition to the typical condition of the postcava, mentioned above, which we may speak of as *Type B*, there are therefore 14 other combinations potentially possible between the right and left postcardinals and the right and left supracardinals in the lumbar region, any one of which may persist in the adult and constitute a variant condition of the postrenal division of the postcava.

These *Types* are as follows (fig. 12):

1. *Type A*, persistence of right postcardinal (*A*) vein.

2. Type *AB*, persistence of right postcardinal (*A*) and right supracardinal (*B*) veins. Right periureteric venous ring.

3. *Type ABC*, persistence of right postcardinal (*A*), right supracardinal (*B*) and left supracardinal (*C*) veins. Right periureteric venous ring.

4. *Type ABCD*, persistence of right postcardinal (*A*), right supracardinal (*B*), left supracardinal (*C*) and left postcardinal (*D*) veins. Right and left periureteric venous rings.

5. *Type ABD*, persistence of right postcardinal (*A*), right supracardinal (*B*) and left postcardinal (*D*) veins. Right periureteric venous ring.

6. *Type AC*, persistence of right postcardinal (*A*) and left supracardinal (*C*) veins.

7. *Type ACD*, persistence of right postcardinal (*A*), left supracardinal (*C*) and left postcardinal (*D*) veins. Left periureteric venous ring.

8. *Type AD*, persistence of right (*A*) and left postcardinal (*D*) veins.

9. *Type BC*, persistence of right (*B*) and left supracardinal (*C*) veins.

10. *Type BCD*, persistence of right (*B*) and left supracardinal (*C*) and left postcardinal (*D*) veins. Left periureteric venous ring.

11. *Type BD*, persistence of right supracardinal (*B*) and left postcardinal (*D*) veins.

12. *Type C*, persistence of left supracardinal (*C*) vein.

13. *Type CD*, persistence of left supracardinal (*C*) and left postcardinal (*D*) veins. Left periureteric venous ring.

14. *Type D*, persistence of left postcardinal (*D*) vein.

With the exception of *Types ABD*, *ABCD* and *BCD*, all of the above-mentioned Types have been observed by us or by others, either in the adult cat or man. Type *ABCD*, however, has been figured by Hochstetter[11] as occurring in Erinaceus europaeus.

It is interesting to note in *Types A*, *D* and *AD* (fig. 12), that the lateral portion of the renal collar (subcardino-supracardinal anatomosis, *Subc. Sprc.Anast.*) does not enter into the formation of the postrenal division of the postcava, and, furthermore that an atypical condition invariably results whenever the postcardinal veins persist in the adult. This further emphasizes the fact, already mentioned, that the right postcardinal vein does not, in the slightest degree, typically enter into the formation of the postcava in the adult cat.

Three other types of variations of the postcava are met with which may result from atypical development of the embryonic veins. These are as follows (fig. 12):

15. *Absence of the Prerenal Division of the Postcava and Substitution for the Latter in the Thoracic Region, of the Right or Left or of Both of the Supracardinal (azygos) Veins, as the Direct Cranial Continuation of the Postrenal Division of the Postcava.*

The postrenal division of the postcava, in such cases, is usually formed by the right (*Type B*), the left (*Type C*) or by both of the supracardinal veins (*Type BC*). It would be quite possible, however, for the postcardinals (*A* and *D*) in the lumbar region also to persist in place of, or in combination with the supracardinal veins (*B* and *C*) but, as far as we are aware, such a condition has not been observed.

[11] Loc cit., Taf. 23, fig. 24.

Cases in which the prerenal division of the postcava (pars hepatica and pars subcardinalis) is wanting, are undoubtedly due to the circumstance that, for some reason or other, the embryonic liver circulation has not been tapped by the right subcardinal vein (figs. 1 and 2). Blood from the liver therefore continues to reach the heart throughout all subsequent stages of development, as in fig. 1, through the *V.* hepatic communis (*V.H.C.*) which serves as the hepatic revehent vein in the adult. In variants of this character in which the postrenal division of the postcava is formed by the supracardinal veins, the renal veins open into the latter and also, on each side of the body, the sex vein opens into the renal vein. This is due to the persistence of the lateral portion of the renal collar on each side of the body, into which the renal and sex veins open, the latter through the postcardinals, and the retention of its connection only with the supracardinal vein. In such cases the lateral portion of the renal collar does not enter into the formation of the postcava and, as the pars subcardinalis of the postcava is wanting, the ventral portion of the renal collar (subcardino-postcardinal anastomosis, *Subc.Pc. Anast.*), has not been formed, or, at least, has not been carried into the adult stage.

The occurrence of variants of this character in the adult cat and man, serves as a complete confirmation of its presence in the embryo and of the morphological unity of the supracardinal system of veins.

16. *Persistence of the Cardinal Collateral Veins* (*The Marsupial Type of Postcava*).

With very few exceptions among marsupials, the postrenal division of the postcava lies ventral to the aorta and its iliac tributaries which is the reverse of the conditions usually observed in placental forms. This has been found by one of the writers[12] to be due to the circumstance that the postcava in the lumbar region is formed in marsupials by a fusion of two veins which takes place ventral to the aorta, and which have been termed the *Cardinal Collateral Veins.*

[12] McClure, C. F. W., *loc. cit.*, 1906.

Veins occupying a similar position to the cardinal collateral veins of marsupials are present in the embryo of the cat. They may form an extensive plexus of vessels which connect with the supracardinals dorsally, and encircle the aorta ventrally between the renal level and the origin from the aorta of the umbilical arteries. In the diagram (fig. 12) only the more caudally situated portion of the cardinal collateral system of veins (*CC*) is shown, where they form a venous ring with contiguous venous channels, on each side of the body, through which the umbilical artery passes. These rings are very constant in character in this location and the *ventral* or cardinal collateral portion of the ring has been found to persist both in the adult cat and man, as the *sole* pathway through which blood can reach the postcardinals or supracardinals, as the case may be, from the external (*E.Il.*) and internal iliac (*I.Il.*) veins. Cases in which the cardinal collateral veins have persisted in the adult are very rare and we know of only three instances, two in the cat and one in man, in which this condition has been observed. In the marsupials, on the other hand, it is the ventral or cardinal collateral (*C.C.*) element of this circum-umbilical venous ring which normally enters into the formation of the postcava in this region, while the dorsal element of the ring disappears.[13]

As far as we have observed, the cardinal collateral veins are very evanescent in character and do not play any especially significant rôle in the normal transformations of the embryonic veins in the cat. For this reason we have omitted them, for the most part, from our diagrams in order to avoid complications and too much detail.

17. *Persistence of the Renal Collar in the Adult.*

We know of three cases in man and none in the cat, in which a complete circum-aortic venous ring, the embryonic renal collar, has persisted in the adult. Now that we know the potential possibilities of the veins in this region, however, other cases of this type of variant will undoubtedly be found, not only in man, but also in the cat.

Instances in the adult in which the left renal vein passes dorsal to the aorta before joining the postcaval vein are also easily ex-

[13] McClure, C. F. W., *loc. cit.*, 1906, p. 203.

plained. This is brought about by the retention of a connection between the lateral portion of the renal collar on the left side and the supracardinal veins, and the atrophy of the anastomosis between the pars subcardinalis of the postcava and the left postcardinal vein (subcardino-postcardinal anastomosis).

The presence of multiple sex veins on one or both sides of the body, or of an anastomosis between the sex veins of opposite sides in the adult, has not proved a difficult matter to interpret, but may best be considered in detail at another time. Variants of this character are largely related, though not always, to atypical conditions of the embryonic subcardinal veins. No instance has yet been found, however, in which the continuous subcardinal channels in the lumbar region, as met with in the embryo (fig. 2), have been carried into the adult stage.

While for convenience of description we may classify the potential variant or atypical conditions of the adult postcava into 17 distinct types, we fully appreciate that combinations of these types may also occur. The circumstance, however, that we now possess a classification based upon a definite ontogenetic plan of the veins, seems to be a distinct advance over our former knowledge, as it not only permits us to interpret the atypical conditions of the postcava thus far observed, but also enables us to predict others which are potentially capable of occurring, and which still remain to be found.

COLOR SCHEME OF FIGURES

Blue: Cardinal System of Veins and their Derivatives.
Red: Subcardinal System of Veins and their Derivatives.
Brown: Supracardinal System of Veins and their Derivatives.
Green: V. hepatic communis and Ductus Venosus Arantii.
Yellow: Subcardino-Supracardinal Anastomosis (lateral portion of Renal Collar) and Renal Veins.
Lavender: Cardinal Collateral Veins.

FIGURES

Figs. 1 to 7, inclusive, diagrams illustrating the development of the veins in the cat (Ventral Views).

Figs. 8 to 11, inclusive, lateral views of reconstructions of the right side of the renal collar and of the right postcardinal, right supracardinal and right sex veins in cat embryos measuring, respectively, 16, 25, 29 and 45 millimeters in length.

Fig. 12. Composite diagram of the embryonic veins of the cat.

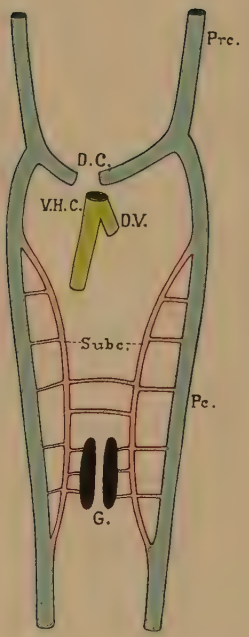
ONTOGENETIC DIVISIONS OF ADULT POSTCAVA (FIG. 7)

Pars Hepatica, P. Hep. Pars Subcardinalis, P. Subc.	Prerenal Division
Pars Renalis, P. Ren. Pars Supracardinalis, P. Spre.	Postrenal Division

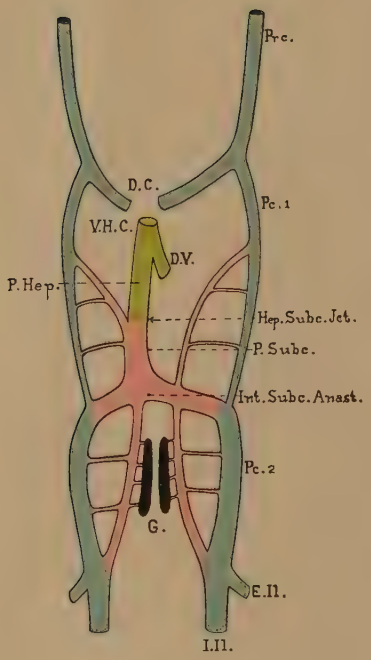
EXPLANATION OF FIGURES

ABBREVIATIONS

A., Right Postcardinal Vein (Lumbar Division)
Adr., Adrenal Vein (Adrenal Organ in fig. 7)
Ao., Aorta
Az., Azygos Vein
B., Right Supracardinal Vein (Lumbar Division)
C., Left Supracardinal Vein (Lumbar Division)
C.C., Cardinal Collateral Veins (fig. 12)
C.Il., Common Iliac Vein
C.J., Common Jugular Vein
C.S., Coronary Sinus
D., Left Postcardinal Vein (Lumbar Division)
D.C., Duct of Cuvier
D.V., Ductus Venosus Arantii
E.Il., External Iliac Vein
E.J., External Jugular Vein
G., Gonad
Hep.Subc.Jct., Hepato-Subcardinal Junction.
I.Il., Internal Iliac Vein.
I.J., Internal Jugular Vein
Int.Subc.Anast., Intersubcardinal Anastomosis.
K., Kidney (Metanephros)
L.In., Left Innominate Vein
P.Hep., Pars Hepatica of Postcava
P.Ren., Pars Renalis of Postcava
P.Subc., Pars Subcardinalis of Postcava
P.Sprc., Pars Supracardinalis of Postcava
Pc., Postcardinal Vein
Pc.1., Postcardinal Vein (Thoracic Division)
Pc.2., Postcardinal Vein (Lumbar Division)
Prc., Precardinal Vein
Prcvi., Precava
R.Col., Subcardino-Supracardinal Anastomosis or lateral portion of Renal Collar in figs. 4 and 5 and on left side in figs. 6 and 7
R.In., Right Innominate Vein
R.V., Renal Vein
Scl., Subclavian Vein
Sprc., Supracardinal Vein
Subc., Subcardinal Vein
Sub. Pc.Anast., Subcardino-Postcardinal Anastomosis
Subc.Sprc.Anast., Subcardino-Supracardinal Anastomosis
S.V., Sex Vein
Ur., *Ureter*
V.H.C., Vena Hepatica Communis

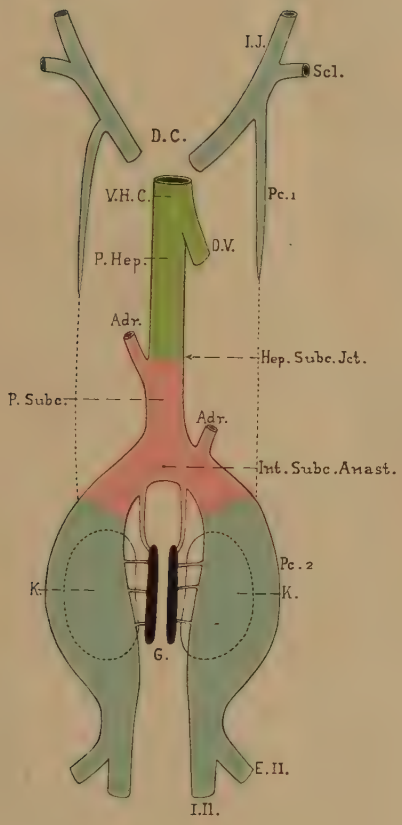


1

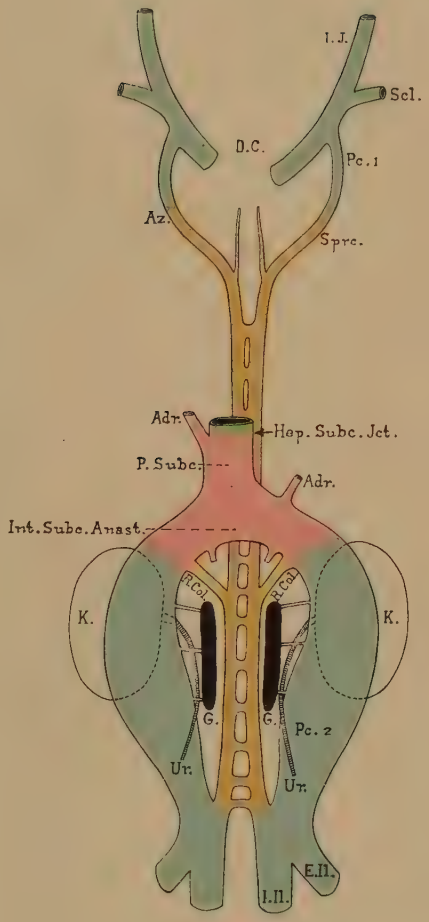


2

PRESS WORK BY FRED'K GOEB

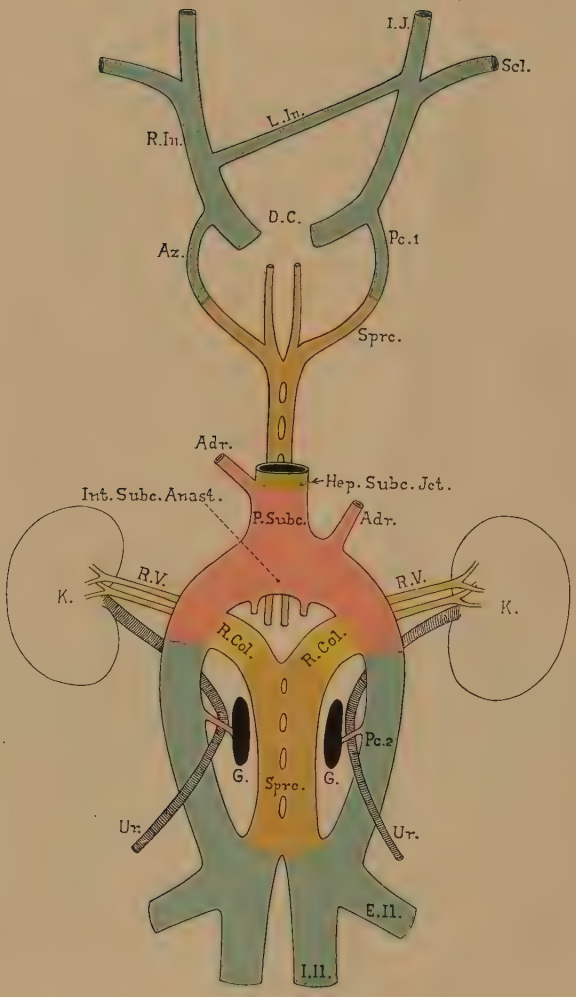


3



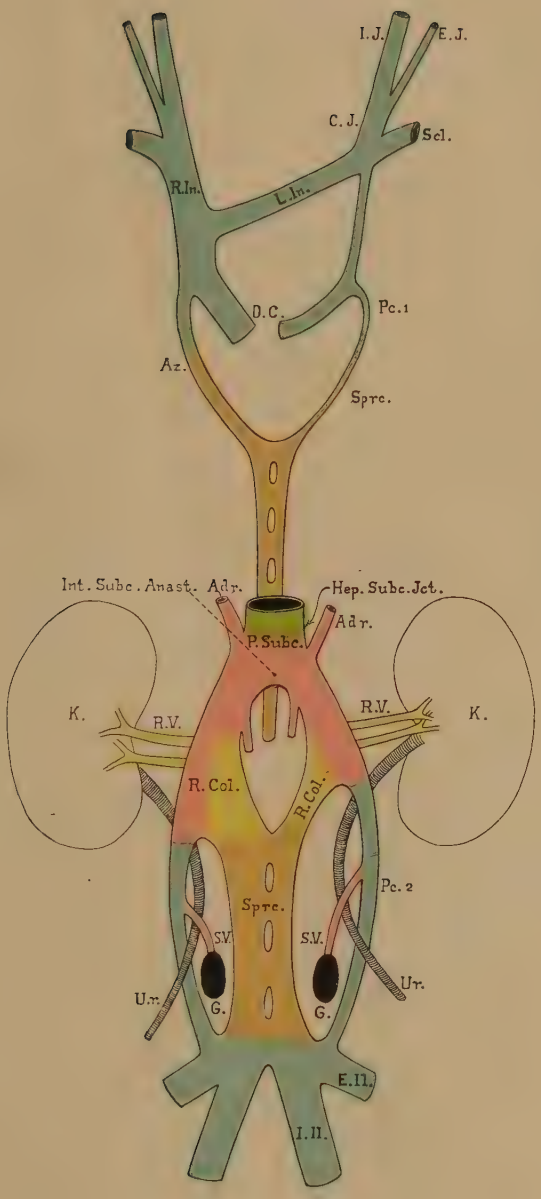
4

PRESS WORK BY FRED'K GOEB



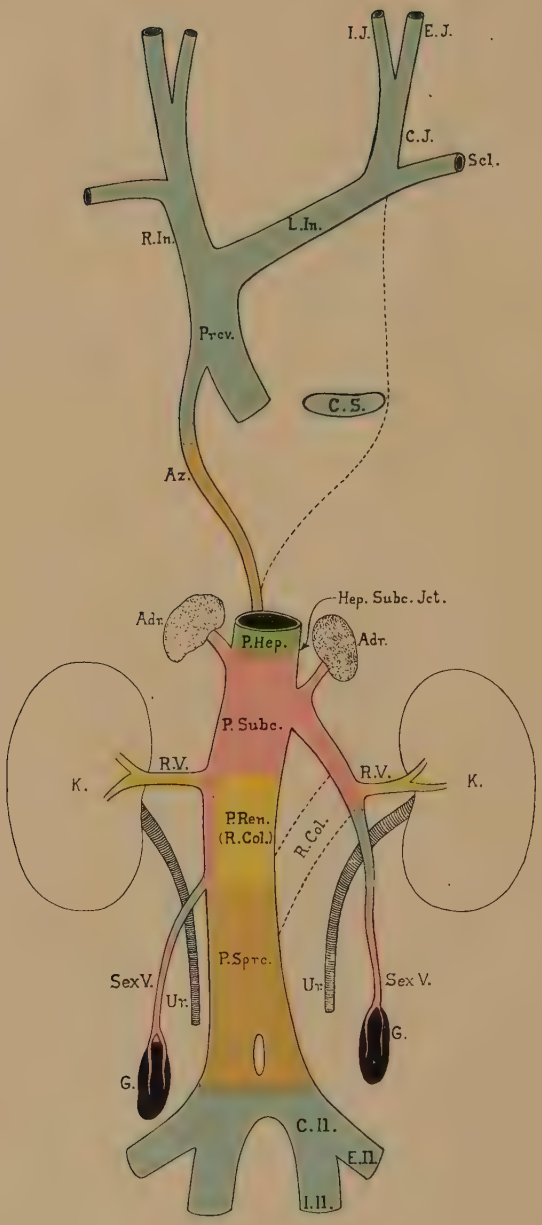
5

PRESS WORK BY FRED'K GOEB

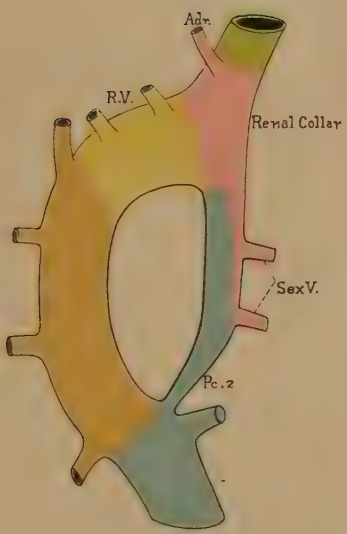


6

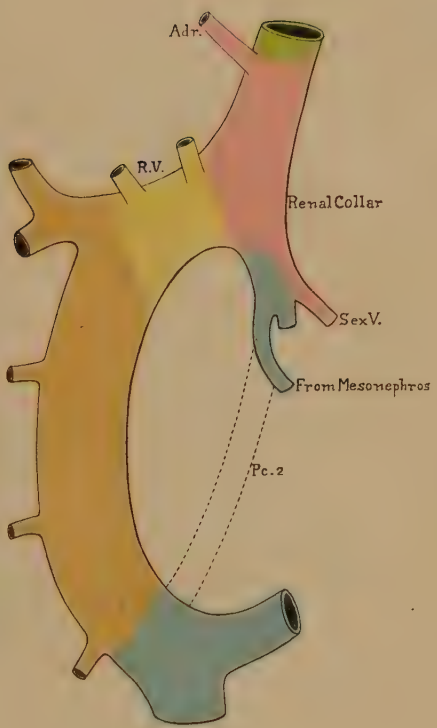
PRESS WORK BY FRED'K GOEB



PRESS WORK BY FRED'K GOEB

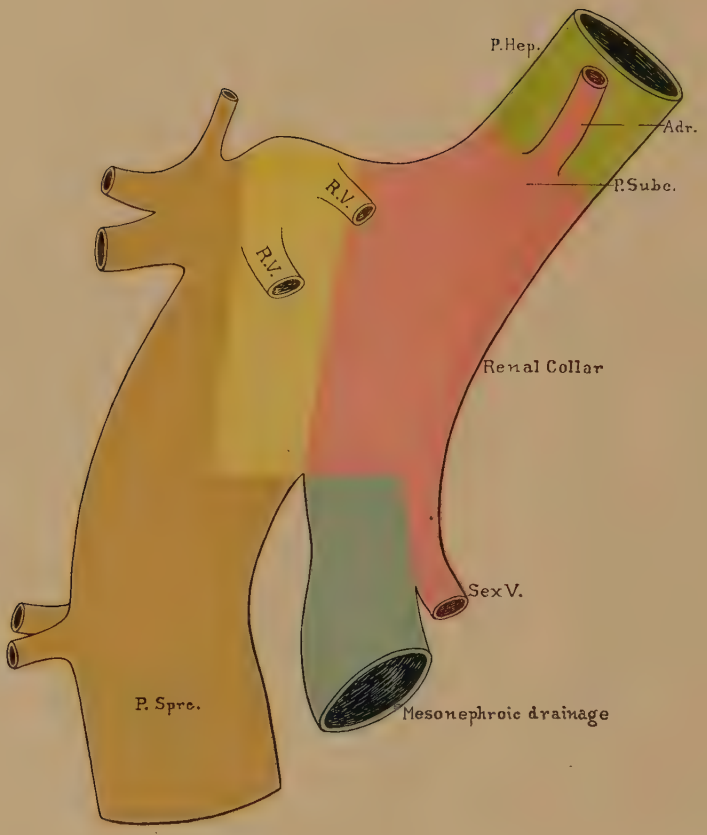


8



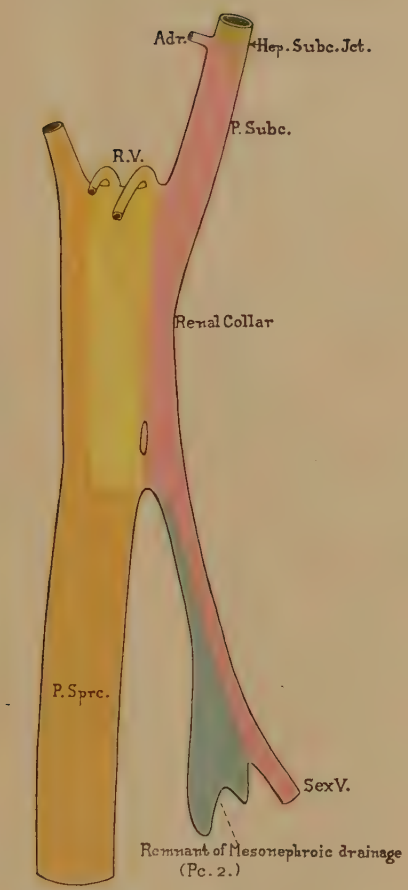
9

PRESS WORK BY FRED'K GOEB



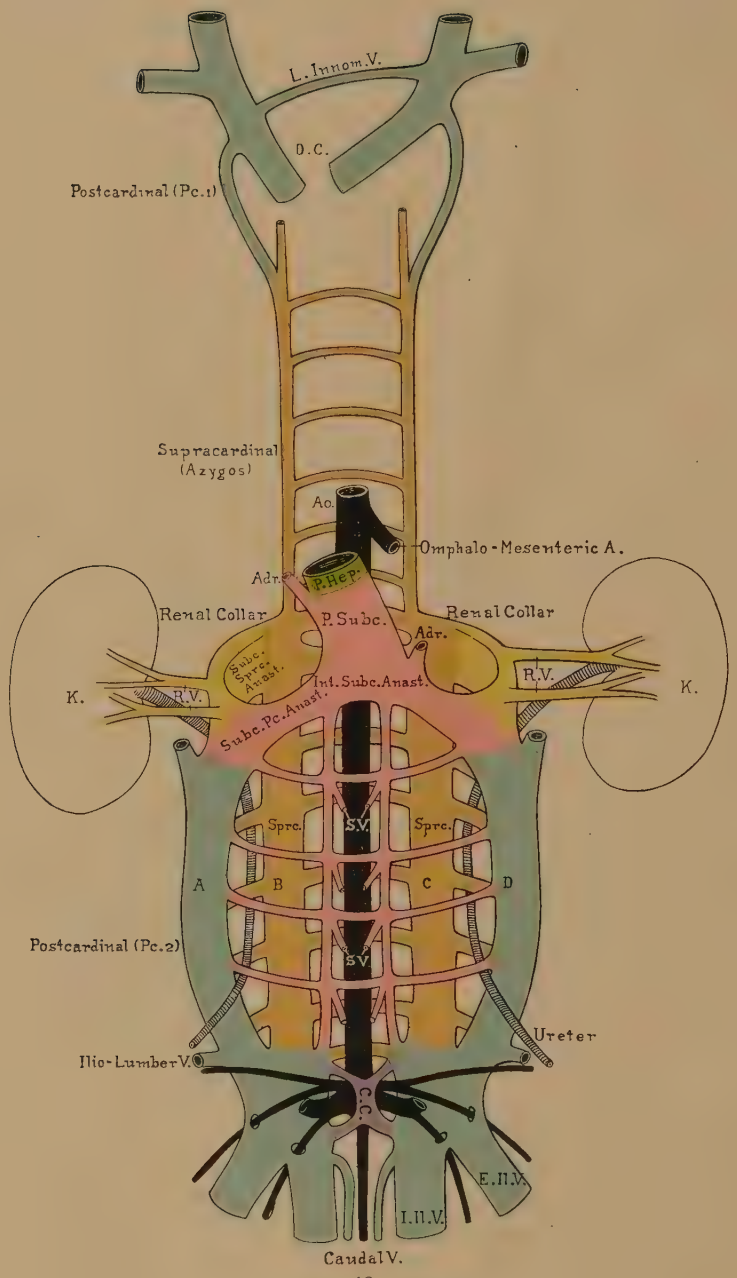
10

PRESS WORK BY FRED'K GOEB



11

PRESS WORK BY FRED'K GOEB



12

30

PRESS WORK BY FRED'K GOEB

Resumen por el autor, E. V. Cowdry,
Peking Union Medical College, Peking, China.

La Anatomía en China.

El presente trabajo es una breve relación de un estudio sistemático sobre las condiciones presentes y necesidades futuras de la ciencia de la Anatomía en China. Después de mencionar los factores principales en la introducción de la Medicina moderna en dicho pais, el autor publica una lista de todas las Escuelas médicas que funcionan actualmente en China, así como lo nombres de los anatómicos que enseñan en ellas, llamando la atención sobre la falta de anatómicos que puedan dedicar todo su tiempo a la enseñanza y la dificultad para obtener material para la disección.

También incluye en su trabajo la lista de socios, constitución y programa de la primera sesión de la Asociación Anatómica y Antropológica de China y discute la influencia de esta Asociación sobre el desarrollo de la Anatomía en el pais mencionado.

Translation by José F. Nonidez
Cornell Medical College, New York

First photograph of the Anatomical and Anthropological Association of China taken at the entrance to the Anatomical Laboratory of the Peking Union Medical College of the Rockefeller Foundation on February 27, 1920.

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, DECEMBER 6

ANATOMY IN CHINA

E. V. COWDRY

Anatomical Laboratory of the Peking Union Medical College of the Rockefeller Foundation

ONE FIGURE

INTRODUCTION

The following agencies are concerned in the development of modern medicine in China:

1. Foreign Missionaries: Most of the pioneer work has undoubtedly been accomplished through the energy and devotion of foreign missionaries who have labored faithfully in the face of prejudice and superstition. In 1913 the China Medical Missionary Association passed the following resolutions which make their purpose quite clear:

1). That in establishing medical colleges and hospitals our sole object is to bring the blessings of healing to the souls and bodies of the people of China, and to give a thorough training in medicine and surgery to young men and women of education and intelligence, enabling them as fully qualified doctors to be of the highest service to their country.

2.) That we have no desire to create permanently foreign institutions, and that our aim and hope is that these medical colleges will, gradually and ultimately, be staffed, financed, and controlled by the Chinese themselves.

3). That we desire to bring our teaching work into line with the regulations of the Ministry of Education, and in all ways to co-operate with and assist the Government of the Republic in medical education, so that a strong and thoroughly equipped medical profession may be established in this great land.

2. Foreign Agencies of Non-Missionary Character: The British Government has materially aided in the development of medicine in South China. It has been particularly successful in securing the support of wealthy Chinese in founding the Medical Depart-

ment of the University of Hongkong. Even the President of China showed his interest in the enterprise by establishing the "Ta Tsung T'ung Ch'uan Hsueh Fei" fellowship for university students. Unfortunately the war has depleted the Colonial Treasury, and, I have been told, the Chinese have shown less interest in the enterprise following the award of the German rights in Shantung to the Japanese. The school buildings, which are up-to-date in every particular, are situated on the side of the mountain and command a fine view of the harbor. The equipment is excellent, the surroundings the finest and most healthful along the South China coast, the facilities for preliminary education good; all that is needed to make the medical department one of the best and most attractive in China is an adequate full time staff. The Professor of Anatomy teaches also clinical surgery and the Professor of Physiology acts as Dean and teaches, in addition, microscopic anatomy. Members of the faculty are allowed to practise as consultants. To keep abreast of recent advances in medical education arrangements must be made for the maintenance of at least three full time men in each of the departments of Anatomy, Physiology and Pathology. The future of this institution is very bright in view of the broad minded attitude of the authorities, that it is the purpose of the Medical School to serve the whole of South China, not merely the Colony and Chinese of British nationality.

The Germans made good beginnings in Shanghai and Tsingtau which were interrupted by the war. The Shanghai School was located in the French Concession and was maintained in close affiliation with a strong engineering department which was financed largely by Krupp interests. After the seizure of the buildings, with part of the general equipment and library, by the French authorities, the teaching was transferred to the Tung Chi and Paulum Hospitals in the International Settlement. Most of the German instructors were deported and the remainder forbidden to enter either of these hospitals, so that at present most of the actual instruction is given to the students by two German doctors in private houses just across the street at Nos. 24 and 25 Burkill Road. There are 120 students and about 40 women

in the Nurses Training School. The institution is now called the Tung Chi Medical School and is apparently being maintained by the Chinese Government. Provision has been made for premedical and engineering work at Woosung and I have been told that the Peking Government has granted a sum of $300,000 for the construction of new buildings for these departments. Most of the information which I obtained at the office of the Commissioner of Foreign Affairs in Shanghai regarding Tung Chi proved to be unreliable and misleading. The other school, in Tsingtau, has been taken over by the Japanese authorities but is not being operated by them.

The Japanese Medical School in Mukden is unquestionably one of the best medical institutions in China. Its strength lies in its full time staff. It publishes a volume of researches each year which will bear careful study. While it is actually owned by the South Manchuria Railway it is nevertheless under strict governmental control. Hospitals are also maintained in nearly all of the large cities by an influential Japanese philanthropic society under the presidency of the distinguished statesman, Count Okuma. In view of the further consideration that a large number of Chinese physicians have received their training in Japan, it is not surprising that the Japanese exercise considerable influence in medical education.

Several American Universities have undertaken medical work in China. In the Yale-Hunan Medical School at Changsha a special attempt has been made to coöperate with the Chinese. This school has made headway in spite of its inaccessibility and the chronically unsettled condition of the province. The land and equipment of the Harvard Medical School in Shanghai was taken over in 1916 by the Rockefeller Foundation.

The China Medical Board of the Rockefeller Foundation is responsible for the most recent developments in medical education. Its purposes are clearly set forth in a letter, under date of March 15, 1915, from Mr. John D. Rockefeller, Jr., to the various missionary societies in Great Britain and the United States as follows:

1. To assist Missionary Societies to strengthen their medical schools and hospitals by providing equipment and other facilities, and by making annual grants, as may be found expedient, for the support of physicians and nurses, selected by the respective Missionary Boards, subject only to the Foundation's approval of the professional qualifications of the appointees.

2. With the consent of the Missionary Boards, to reorganize and expand existing medical schools, with their hospitals, and to support these, wholly or in part, from its own funds.

3. To aid other medical schools that are not strictly missionary.

4. To establish, equip and support new medical schools and hospitals. In choosing its agents, physicians and nurses for independent schools or hospitals, the Foundation will select only persons of sound sense and high character, who are sympathetic with the missionary spirit and motive, who are thoroughly qualified for their work professionally, and who will dedicate themselves to medical ministration in China. Beyond these qualifications, the Foundation cannot properly impose tests of a denominational or doctrinal nature, such as are deemed desirable by Missionary Boards for their own medical missionaries or agents.

The Board originally planned to establish two new medical schools, one in Peking and the other in Shanghai, but has recently decided to devote all its energies to the foundation and mainteance of one really first class school in Peking. To this end $7,000,000 have already been expended on buildings and equipment. The yearly budget is a large one owing to the fact that the entire staff is on a full time basis. It already amounts to about $750,000 per annum and will probably reach a million in the near future. The China Medical Board has also contributed generously toward the assistance of other medical schools and hospitals. Up to the end of 1918 payments totalling $676,889.70 were made to thirty-one institutions.[1]

3. The Chinese themselves.[2] The work of the Chinese Government should be given careful attention. The Army Medical

[1] Roger S. Greene, The Rockefeller Foundation in China, "Asia," November, 1919.

[2] "The Chinese government has discovered that it has a saving's account with a credit balance of five million dollars in the Russo-Asiatic Bank. The amount was placed there by the old Imperial Board of Education in Manchu days, when the department of education was one of the wealthiest in the government. Owing to the confusion incident to the change of regime, and the disappearance of the officials who deposited the money, the account was entirely lost sight of." (North China Star, May 13, 1920.)

School, the Naval Medical School, the National Medical College and the five Provincial Medical Schools constitute a creditable foundation on which to build. They represent a conscientious attempt on the part of the Chinese to assume responsibility for their own needs in the way of medical education. The late President, Yuan Shih-Kai, declared, that "For a country to be strong and prosperous it is essential that its citizens be healthy."

It is important that these medical schools should grow rapidly and become the back bone of the nation. Up to the present they have received very little foreign support and it is doubtful whether they will accept any. The Director of one of them told me that open coöperation with a foreign institution might have a bad effect upon his annual budget (owing to antiforeign feeling). Some medical missionaries, on the other hand, take but little interest in Chinese schools because they see in them no opportunity for evangelistic work. One doctor dismissed the question by saying that "it is difficult to coöperate with them because they are only heathens any way."

The case of the Kung Yee Medical College is instructive. This school belongs to a group of Chinese in Canton. It has excellent buildings in a fine location, and, until recently, had a fairly complete staff. The instruction given is of a relatively high grade. The educational policy of the institution was under the control of an executive committee in which foreign influence predominated. A proposal was made to become affiliated with the Canton Hospital (Missionary) which was apparently acceptable to all parties. At the last moment, unexpectedly and in an unconstitutional way, the Chinese members of the Kung Yee Society refused to ratify the agreement. The missionary element in Canton is almost unanimous in declaring that they can have no more confidence in the Kung Yee Society and that further negotiations are impossible. Affiliation has now been effected between the Canton Hospital and the Canton Christian College.

The Chinese schools have been unable to keep pace with the rapid advances in the foreign controlled institutions, which tend to attract the best students. With a depleted treasury the Chi-

nese may prove reluctant to make large expenditures to accomplish something which the foreigners will do for them gratis. Very skillful and diplomatic assistance is called for. As a rule the buildings and equipment are adequate for the present needs. It might be possible, however, to improve the library accommodations and to strengthen the teaching staff. The addition of one or two really well trained Chinese, who have travelled abroad, with a liberal salary guaranteed, to one of these colleges would soon react beneficially upon the whole institution. It is also desirable that members of the staff, already under appointment, should be given an opportunity to travel. Above all, research work should be encouraged. It might be a good idea to try the European policy of offering prizes and medals for the best work done. Perhaps the President of China would himself consent to make the awards.

The underlying difficulty, however, is the deeply rooted conviction, handed down for forty centuries, that the medical profession is but a fourth or fifth rate occupation. The sentiment of a nation like China cannot be changed over night nor yet in fifty years' time, but a beginning has been made in Peking, for example, where the splendid buildings of the Rockefeller Foundation will surely lead people to doubt whether, after all, the medical profession is so very degrading. The raising of a despised trade to the level of a dignified profession requires long and sustained effort throughout the country, but until it has been accomplished, we cannot hope for any real development of modern medicine in China.

The people generally look upon disease and sickness in an apathetic and fatalistic way, believing that it is a visitation of providence in punishment for their transgressions, or at any rate that it is the will of God, as our forefathers thought in Europe several hundred years ago and some continue to think. While such views prevail there can be no real progress. This tendency to shift the responsibility from their own shoulders is characteristic of the Chinese in all their dealings, and will be very difficult to correct. They are handicapped also by certain customs like foot binding, and the binding of the breasts in young women

before marriage, which they still practice blindly, having forgotten their origin and not troubling to ask the why or the wherefore.

Efforts to introduce modern medicine in China will be unavailing unless a nation-wide campaign is carried on by Chinese and foreigners alike for the education of public opinion. At present the graduates of the schools which have been established are regarded with indifference or active distrust by the vast majority of their countrymen. It is particularly difficult for those of them who have to go into the interior, away from the thin film of foreign influence, to live up to their new ideals, in the face of universal incredulity and without sympathy or assistance of any kind. A beginning is being made from several angles without proper coördination and on a very small scale. Professor John Dewey of Columbia University is perhaps doing more than any one man to divert the funds squandered by the millitarists into educational channels and to lead the mass of the people to see themselves as others see them and to assume full responsibility for the orderly development of their own lives. During vacations some of our students make a practise of giving public lectures on hygiene and preventive medicine; but what are one or two among so many? I am thoroughly in sympathy with Doctor Peter's public health campaign but I should like to see it on a much larger scale like the world-wide demonstrations of the International Health Board. The Chinese people are not devoid of business instinct and I am inclined to think if it were demonstrated to them repeatedly how much may be actually gained by improved sanitation and through an intelligent appreciation of the principles of hygiene, that they would not be unresponsive. Every means of publicity should be utilized and the vernacular press pushed to the limit in a concerted attempt to educate the general masses of the population to a realization and appreciation of the good work which is being done in the medical schools and hospitals throughout China, emphasis being placed upon those under Chinese control.

At the same time wealthy merchants and business men should face their duty to their country. It would not be at all a difficult matter to select a group of five or six Chinese in each of the great

cities of Kankow, Shanghai and Canton who could, with but little personal sacrifice, establish and maintain a medical school on an equally efficient and elaborate scale to that founded through the generosity of an American citizen in Peking. The example has been set and I think that we can count on its being followed.[3]

A list of the medical schools with the names of the anatomists follows. Those marked with * were personally visited.

Canton

1. *Kwangtung Provincial Medical College.
2. *Hackett Medical College for Women.
   Harriett M. Allyn (part time)
   S. W. Kwan (part time)
3. *Kung Yee Medical School
   D. J. Todd (part time)
   J. A. Hofmann (part time)
   John Kirk (part time)
   Wong Tak Kwong (part time)
   Wong King Yip (part time)
   Ch'ui Kam Ch'i (part time)
4. *Kwang Wha Medical School
5. *Ecole de Medicine Franco-Chinoise de Canton
   Gilbert Desrallons (part time)
   P. Tsoi (part time)
6. Liang Yueh Medical College
   Chee Sek-chong
   Leung Kin-Cho (part time)

Changsha

7. Hunan-Yale Medical School
   T. C. Lieu
   A. S. Crawford (part time)
   J. W. Williams (part time)
   P. C. Chu (part time)

Chengtu

8. West China Union University
   H. L. Canright (part time)
   W. R. Morse (part time)

[3] "In connection with the proposed organization of a University in Amoy, for which $4,000,000 has been donated by Mr. Chen Kia-Keng, a wealthy retired merchant in the Straits, it is now reported that another rich Straits merchant, Mr. Wang Yig-Chu, has given a further sum of $3,000,000 for the establishment of a medical college in the University." (China Medical Journal, 1920, vol. xxiv, p. 216.)

Foochow

9. Union Medical College
   Jesse Gossard (part time)

Hangchow

10. Hangchow Provincial Medical College
    Li Ding
11. Hangchow Hospital and Medical Training College
    Tsu Peh Long
    Dzen Ven Dah (part time)

Hongkong

12. *Hongkong University
    H. T. Earle (part time)
    Kenelm H. Digby (part time)
    C. C. Wang (part time)

Mukden

13. *South Manchuria Railway Medical School
    K. Shiino
    K. Kudo
    T Mikami
14. *Union Medical College
    R. H. Mole (part time)

Nanchang

15. Nanchang Provincial Medical College

Paotingfu

16. *Chihli Provincial Special Medical School
    Chang Peh Ching
    Tien Yuen Chin (part time)

Peking

17. *Army Medical School
    C. P. Ch'ang
    W. S. Kuei (part time)
    C. Y. Hei (part time)
18. *Government Medical School
    K. Ikegami
    Dr. Futamura
19. *North China Union Medical School for Women
    Ethel Leonard (part time)
    Li Pau Chen (part time)
20. *Peking Union Medical College of the Rockefeller Foundation
    E. V. Cowdry
    Davidson Black
    S. R. Detwiler
    R. S. Stone
    Paul H. Stevenson

Shanghai

21. *St. Johns University (Pennsylvania Medical School)
    E. M. Merrins
    Dr. Lincoln (part time)
    Dr. Yin (part time)
    W. S. New (part time)
22. *L'Aurore University Medical School
    Dr. Florence
    R. P. Hernault
23. *Tung Chi Medical College (being a continuation of the former German Medical School)

Soochow

24. *Soochow (Kiangsu) Provincial Government Medical College
    Sah Fou-Zien
    Tsu Ho Yung

Tientsin

25. *Naval Medical School

Tsinanfu

26. *Shantung Christian University (1918–19)
    R. T. Shields
    L. M. Ingle
    Wu Djao Hsiang
    Wang Hwei Wen

BUILDINGS AND EQUIPMENT

The anatomical laboratories in China vary from a single bare room to costly buildings fitted with every convenience. The anatomical laboratory of the Peking Union Medical College, here illustrated is certainly the most elaborate. The anatomical laboratories of the Japanese Medical School in Mukden are housed in a plain but substantial building and are fully equipped with everything necessary for teaching and research. The University of Hongkong has good reason to be proud of the School of Anatomy erected in a splendid location, high up on the mountain side largely through the generosity of the late Mr. Ng Li Hing, one of the leading merchants of the colony. Mention should also be made of the new anatomical laboratory of L'Aurore University, the austere simplicity of which is quite pleasing. Some of the Chinese institutions are a strange mixture of the old and the new. Occasionally one may see mirrors placed at the top of a flight of

stairs, and in other strategic positions, in order to turn back the evil spirits and in this way make the place more healthy.

I shall not take space to describe the smaller laboratories, some of which are very unprepossessing, except to say that good work may be done with very meagre equipment if it is gone about in the right way. One becomes very tolerant in China. From modest beginnings great developments may be expected. The mere rudiments of anatomy properly or even indifferently taught will serve to indicate the fallacy and the danger of native Chinese medicine. Our policy is to encourage and stimulate, not to go away with the feeling that the conditions are hopeless.

Both large and small institutious suffer from lack of library facilities. At present there is no library in the whole of China to which one can refer for back numbers of standard European journals. Some are to be found in Peking (where the library of the Peking Union Medical College is being rapidly enlarged) one or two in Shanghai and a few more in Hongkong. Tung Chi has a few valuable sets of German journals. Under these conditions teaching is hampered and research work is set aside because it is not altogether satisfactory to have to devote time and energy to a problem which may already have been solved by some one else. Dr. Greenman's policy of the wide distribution of the Wistar journals in China is most helpful and constitutes an important step in the right direction. The relatives of the late Mr. Andrew Carnegie could make no more fitting memorial to him than the establishment of a real library in China, free to millions of people.

STAFF

The twenty-six medical schools of China number only about two dozen teachers who are able to devote all their time to anatomy. These teachers are certainly overworked but their plight is not so bad as those who have to teach a whole variety of subjects and sometimes have to attend to hospital and, more rarely, to private practice in addition. Often the instructors are so busy that they have neither the time nor the energy to settle down and do one thing well, with the result that makeshifts and time saving devices are resorted to. Chinese medical schools are

inferior to those of Japan in this respect. It is not that those in authority fail to appreciate the gravity of the situation; they are simply unable to remedy it through lack of funds. In several schools, however, one cannot help feeling that some of the money invested in buildings could more profitably have been spent on the staff. The teachers suffer with the students. They leave promising, perhaps remunerative, careers behind them and come out to China young and enthusiastic. They have innumerable demands upon their time and energy, they do really pioneer work in isolated stations, and become, to some extent, 'Jacks of all trade.' Middle age finds them often with large families, but without that modern prerequisite—a specialty—so that they are just a little out of the running for positions in the modern and up-to-date medical schools which are bound to spring up. It is essential that anatomists and others shall have a certain amount of leisure time to plan their teaching, to keep up with world progress and to engage in research. Unfortunately it is always easier to secure funds for buildings and equipment, which represent something tangible and permanent, than for the salaries of teachers and investigators. With patience, however, the condition in China will slowly improve.

TEACHING

With foreigners of so many nationalities it is not surprising that the methods of teaching anatomy should be varied. Perhaps the most pernicious system is as follows: The foreign instructor, who speaks not a word of Chinese, comes in and makes anatomical drawings on the blackboard and labels the parts. A Chinese interpreter then makes his appearance and translates the terms into Chinese. When the students return next day they are required to repeat the drawings from memory. They have no practice in dissecting, even on animals, and accordingly have to learn how to use their instruments upon the living subject, sometimes with disastrous results. One of my questionnaires was answered as follows:

"We don't teach histology, embryology, comparative anatomy, as our scope is to form practitioners only. Besides, the intel-

lectual standard and scientific previous education of our scholars are not high enough to allow us to emphasize pure science."

The greatest stumbling block in the teaching of anatomy, next to inadequacy of staff, is the difficulty of obtaining human material for dissection. To the best of my knowledge only the Japanese Medical School at Mukden and the University of Hongkong have a sufficient and regular supply of bodies on which they can rely. Only twelve of the twenty-six medical schools offer regular courses in human dissection. In Peking we have only been able to secure four bodies in the last year and a half. When the first entered the building all our servants left us immediately and we had some difficulty in replacing them. The police and the authorities generally are not sympathetic, and there seems to be but little hope of obtaining sufficient material in Peking for sometime to come. In the provinces some of the schools obtain material from executions but this source is sporadic and unsatisfactory. It is interesting that in Japan, where the worship of ancestors is also prevalent, bodies may be obtained more easily than perhaps anywhere else in the world (Cowdry '20, p. 72).

The regulations regarding dissection of the Chinese Government are as follows, quoting the translation given in the China Medical Journal:

Order of the Board of Interior No. 51, November 22, 1913:

Article I. A physician, in case of death from disease, may dissect the body and inspect the diseased part to determine (examine) the origin of the disease, but he must first obtain the consent of the relatives of the dead person and clearly inform the local magistrate before proceeding to dissection.

Article II. The police and inspectors, in case of mysterious death, the cause and origin of which cannot be accurately ascertained without dissection, may appoint a physician to dissect said corpse.

Article III. The bodies of all those meeting death by punishment or dying in prison from disease, without relatives and friends to claim their bodies, may be given by the local magistrate to a physician for dissection, to be used for the purpose of experimentation in medical science, but after dissection the body must be sewed up and buried.

Article IV. If any are willing for the benefit of science to offer their bodies for dissection and leave word to that effect before death, they may do so, but the whole body must be sewed up and returned to his or her family after dissection.

Article V. These regulations are in force from the day of their proclamation.

Supplementary order of the Board of Interior, No. 85, April 22, 1914.

Article 1. All medical colleges and hospitals, which are proved to be in good condition by the local authorities and recognized beforehand by the Board of Education or established by the public, shall be allowed to perform dissections.

Article 2. According to Acts No. 1 and 4 of the General Laws this may be enforced and the medical men allowed to perform post-mortems as soon as the consent of the family is obtained. (During summer this may be done immediately after reporting to the local authorities.)

Article 3. When the colleges and hospitals mentioned in Art. 1 of By-laws apply for any dead body from the local authorities the following rules must be observed:—

i. Proper letters with official seals are needed on both sides—local authorities and the college—when dealing with any deceased criminal or deceased prisoner. Any private medical college recognized by the Board of Education may also apply in similar manner.

ii. Special certificates shall be made by the judicial authorities of the local government, and the same shall be given at the time when dead bodies are issued to the medical colleges. After examination the certificates shall be kept in the college until the end of the month when they shall be returned to the local authorities so as to enable them to preserve records. There shall be no need to send these to the prisons.

iii. The name, age, district, and number of the dead person shall be noted in the certificate, which shall be properly dated with official seals by the local government before the same is sent. The college receiving the corpse shall keep a copy of the name, age, date, etc., so as to facilitate examination when required.

Article 4. A certificate of death by a qualified medical man shall first be sent to the local authorities before a post-mortem examination is allowed on certain persons, who have not died in a hospital as mentioned in Art. 4 of General Laws. After examination a report shall be submitted to the local authorities for reference.

Article 5. With the exception of Arts. 1 and 4 of General Laws any or many parts of a dead body dissected may be retained, if such are necessary for medical demonstration. This may be done according to Art. 3 of General Laws.

Article 6. When any or many parts have been removed from a dead body for medical demonstrations, the rest of the corpse shall, if possible, be sewed up according to Arts. 3 and 4 of General Laws. (Dead bodies mentioned in Art. 3 of General Laws which are supplied to medical colleges shall be treated in the same way.)

Article 7. A dissected body after being sewed up shall be returned to the family if possible. If unclaimed it shall be buried by the college which has dissected the body. After the funeral, a sign shall be shown

on the tomb where the deceased has been buried. (Any deceased person having no family as mentioned in Art. 3 of General Laws may be taken to a crematorium by the medical college and cremated if necessary. After burning, the ashes of the deceased shall be gathered and buried, and proper signs shown on the tomb. This shall be duly reported to the local authorities.)

Article 8. All medical colleges shall report yearly the number of dead bodies dissected, to the police court if at Peking, and to the local authorities at other places, in order to facilitate reporting to the Board of Interior for the preservation of the records.

Article 9. These By-laws may be revised at any time with a view to improvement.

Article 10. These By-laws shall be enforced on date of promulgation.

The chief difficulty is that these regulations are interpreted to mean that the written consent of the individual before death must be supplemented by the sanction of the relative, which it is almost impossible to obtain.

Owing to the pressure of other duties, the teachers are often unable to properly adapt their methods of teaching to the lack of human material. They soon come to rely upon the use of anatomical models made in Europe or Japan and give up their efforts to obtain bodies for dissection; for it takes a lot of time and energy to cultivate the authorities, to drink endless cups of tea, to make petitions for favorable legislation and arrangements for executions. Yet it is possible, with a little care, to give the students an idea of the science of anatomy without the aid of human dissection. It is usually quite a simple matter to obtain a skeleton from abroad or even in China. For instance, the students at Paotingfu have, through their own initiative, amassed quite an interesting and useful collection of bones from the graves in the vicinity. Living models should be used extensively. The students should be obliged to make a careful and complete dissection of some mammal comparing the structure carefully with that of man. In the south of China advantage may be taken of the monkeys which are sold as pets, for from two to five dollars a piece.

Lack of time and insufficient laboratory equipment are responsible, in some cases, for a rather low standard of work in histology, embryology and neurology.

While the teaching in the better schools is worthy of the highest praise, there is a general tendency, which I have already noted in the case of the Japanese medical schools, to give too many lectures and too little laboratory work. It is true that the cost of laboratory furnishings may be a certain deterrent; but, on the other hand there is the time factor. It is more wearing on the teacher to give one hundred lectures than to teach in the laboratory for the same length of time. The proportion of lectures to laboratory work in some of the principal colleges is given in table 1.

TABLE 1

Proportion of lectures to laboratory work in anatomy[4]

COLLEGE	LECTURES	LABORATORY
	per cent	*per cent*
Army Medical School	83.0	17.0
Chekiang Provincial Medical College	86.6	13.4
Chihli Provincial Medical College	91.3	8.7
École de Medicine Franco-Chinoise	100.0	0
Hackett Medical College	35.8	54.2
Hangchow Hospital and Medical Training College	80.0	20.0
Hunan Yale Medical College	32.7	67.3
Japanese Medical School, Mukden	50.0	50.0
Kiangsu Provincial Medical College	71.4	28.6
Kung Yee Medical College	36.9	63.1
Liang-Yueh Medical College	80.0	20.0
Peking Governmental Medical College	70.4	29.6
Peking Union Medical College	10.0	90.0
West China Union University	40.5	59.5

This high percentage of lectures is very bad for the students. It means that most of their information comes to them second hand, predigested in the mind of the lecturer, and that they are, to a large extent, robbed of the incentive and privilege of making observations for themselves and of learning how to make logical deductions therefrom. This method of training does not teach the student self reliance, which is particularly necessary in China, where, after graduation, he is often placed entirely on his own resources without sympathetic and stimulating help from those around him.

[4] Unfortunately information has not yet been received from St. John's University.

In some colleges the time devoted to anatomy is altogether too long. I learn for example, from the Kung Yee Medical College, in answer to my questionnaire, that the students suffer 1178 hours of instruction in anatomy. Long hours with lack of time to think and of opportunity to pursue work along special lines, chosen by the students themselves, is deadening. It usually fails to create in the student a thirst for knowledge and a desire to help himself.

There is one more consideration in the training of the student which should, in my opinion, receive attention in view of the fact that it is the purpose of the medical schools to turn out men of strong character, not merely physicians skilled in their professions. Reading through the printed announcements of the various colleges we meet with catagorical prohibitions relating to the conduct of the students. A merely negative attitude of this kind does not itself tend to strengthen character. When all restraint is removed and the student leaves the college a reaction is in danger of setting in. It is surely through education, teaching that what is right is also expedient, that the best results are to be obtained.

The standard of instruction has unquestionably been raised by the action of the Council on Medical Education (1915) in urging the various medical schools to meet the following requirements necessary for admission to "Grade A:"

1. Course of Instruction. That the course of instruction shall extend over a period of five years of, at least, 32 weeks each.

That the text books used and the instruction given shall be equivalent to that in European and American schools.

That human dissection, and complete courses in laboratory work, shall be included in the curriculum.

2. Entrance Requirements. That the standard of admission shall be graduation from a Middle School as defined by the Educational Association of China, and, in addition, at least one year of preliminary work including laboratory courses in physics, chemistry and biology; this preliminary or "pre-medical" year being arranged to supplement the preparatory instruction already given in Middle Schools.

BOSTON MEDICAL
MAY 9 1921
LIBRARY

3. Hospital Year. That before receiving a medical degree, students who have completed the five years of regular instruction, shall spend one year as interne in an approved hospital, or in some other line of special medical work, at the conclusion of which the applicant for a degree shall present a thesis acceptable to the faculty of the medical school.

4. Degrees. That only students who have met the entrance requirements, have completed the regular course of instruction in an approved school, and at the termination of the hospital year have presented an acceptable thesis shall be entitled to receive a medical degree.

5. Staff. That the minimum staff shall be ten men on the field giving full teaching time. To provide for furloughs, language study, etc., this requirement means a staff of at least fifteen fully qualified teachers either foreign or Chinese.

6. Equipment. The school must be adequately equipped with modern appliances for instruction, properly equipped laboratories in Chemistry, Pathology, Bacteriology, Anatomy, and Clinical Microscopy, and must be maintained on an efficient basis. The scientific instruments and apparatus shall be sufficient to permit students to do individual work.

7. Hospital Facilities In connection with each medical school and under control of the faculty, there shall be one or more hospitals suitable for teaching purposes, each hospital to have at least 100 beds. One or more daily dispensaries should be connected with the college hospital.

8. Curriculum. The schools approved by the C. M. M. A. shall meet the curriculum requirements which the Council on Medical Education will prepare in detail upon the lines already suggested.

While travelling from college to college one is impressed with the relatively small attendance. Very few of the colleges are filled to capacity. This is to be attributed to the fact that the science of medicine does not appear to be very attractive to Chinese students, rather than to the lack of premedical training which may easily be secured in Peking, Shanghai, Hongkong and the larger cities. It is difficult to ascertain the maximum number

of students which may enter the medical schools of China each year, because in the poorer schools, where but little attention is given to laboratory work, almost any number may attend the lectures. The Chinese Government Schools are certainly much better attended than the missionary establishments, perhaps because their entrance requirements are less exacting. Table 2 will show how meagre is the supply of doctors which is being trained to supply the needs of 400 million people. The entering classes of 16 colleges combined only contain 355 students. Contrast this with the conditions following the war in the United States and Canada. The University of Toronto, for instance, has an entering class in the medical school of 416 students to supply the needs of a country with a population of about 8 millions, already quite well stocked with doctors.

During the last few years the following medical schools have been closed, partly through a desire to concentrate activities and thus increase the standard of work:

Harvard Medical School, Shanghai.
Women's Medical School, Soochow.
University of Nanking Medical School, Nanking.
Union Medical College, Hankow.
German Medical School, Tsingtau.
Provincial Medical School, Wuchang (?).

The following resolution of the Council on Medical Education (1920) seems to forecast the closure of other schools through lack of adequate support:

> That in view of the difficulties hitherto experienced in securing adequate staff and support for the medical schools at Changsha, Chengtu, Mukden and Foochow, the Association would commend to the incoming Council the careful consideration of the following question, in consultation with the missions interested in the development of those schools:
>
> *a.* Whether steps can be taken to meet the urgent need of a broadened basis of support for the Medical School at Changsha, by the provision of an adequate endowment, or by the full co-operation of missions working in that area, in view of the fact that should this not be obtained, the continued existence of the school will prove impracticable.
>
> *b.* Whether the Missions at work in Southwest China can set aside a sufficiently large staff of efficient teachers to enable the West China

Union University Medical Department to approximate to the C. M. M. A. minimum requirements.

c. Whether some measure of co-operation between the Union Medical College, Mukden, and the School of Medicine, Tsinan, could not be satisfactorily arranged.

d. Seeing that the Fukien Christian University is not intending to develop a medical department, as the Council is informed, should not the authorities of the Fukien Medical College be urged to reconsider the continuance of that school?

TABLE 2

Actual attendance and teaching capacity

COLLEGE	ACTUAL NUMBER IN FIRST YEAR	MAXIMUM NUMBER
Army Medical School	51	85
Chihli Provincial Medical College	42	45
École de Medicine Franco-Chinoise de Canton	25	
Hackett Medical College	14	
Hangchow Hospital and Medical Training College	21	50
Hangchow Provincial Medical School	30	80
Hunan Yale College of Medicine	7	
Japanese Medical School, Mukden	57	60
Kung Yee Medical College	20	40
Kwangtung Provincial Medical College	25	50
Kwang-Wha Medical College	14	25
Liang-Yueh Medical College	8	
Peking Union Medical College	7	25
Shantung Christian University ('19–20)	10	30
Soochow Provincial Medical School	14	60
West China Union Univesrity	10	

Women of the better class are gradually becoming interested in medical education and the outlook for the future is good. At least six medical schools welcome them in China, whereas in Japan they are permitted to enter only one, and that of distinctly inferior grade. Notwithstanding the fact that a beginning has been made in coeducation, and the initial prejudices of a very conservative race partly overcome, there is a strong movement for the strengthening and maintenance of at least two medical schools exclusively for women, one in the North or Central China and the other in the South, which shall be in large measure staffed and controlled by women. The wisdom of introducing

a system of education into China which has not proved eminently satisfactory in Europe or America is doubtful. It is fair, I think, to say that in the United States the best students seek to enter Hopkins, Chicago and other coeducational institutions in preference to the women's medical colleges, in which it has never been possible to establish an equally high grade of instruction. If China is to keep abreast of the times, it is essential that her women shall be encouraged to come out and exercise their influence, not to retire into the seclusion of feminine institutions.

RESEARCH

It is not an easy matter to carry on research work in China. One of the greatest obstacles is geographical isolation enhanced by insufficient library facilities. The country is so vast and the number of anatomists so small that each must rely almost entirely upon his own resources. In order to bring together men and women of like interest and to create a stimulating intellectual atmosphere an "Anatomical and Anthropological Association of China" was formed at a meeting held on Thursday afternoon, February 26th, 1920, in the anatomical laboratory of the Peking Union Medical College, as follows:

HONORARY MEMBERS

Aleš Hrdlička, *U. S. National Museum*, Washington, D. C.

ACTIVE MEMBERS

Andersson, J. G., Peking, (*Councillor*)
Black, Davidson, *Department of Anatomy, Peking Union Medical College*, (*Councillor*)
Boring, Alice M., *Department of Biology, Peking Union Medical College.*
Britland, A. J. D., *Peking Union Medical College Hospital.*
Brubacker, A. F., Lia Chow, Shansi.
Chang, P. C., *Department of Anatomy, Chihli Provincial Medical School*, Paotingfu.
Chen, S. P., Tsung Pu Hutung, Peking. (*Councillor*)
Cowdry, E. V., *Department of Anatomy, Peking Union Medical College*, (*President*)
Cowdry, N. H., *Department of Anatomy, Peking Union Medical College.*

CHUAN, S. H., *Director, Army Medical College, Peking,* (*Councillor*).
DANTON, G. H., *Tsing Hua College,* Peking.
DIGBY, KENELM, H., *Department of Anatomy, University of Hongkong,* Hongkong.
DITTMER, E. G., *Tsing Hua College,* Peking.
DOBSON, R. J., *Peking Union University,* Peking.
EARLE, H. T., Dean of the Medical Faculty, *Victoria University,* Hongkong, (*Councillor*).
FAUST, F. C., *Department of Pathology, Peking Union Medical College.*
GRAY, DOUGLAS, *Physician to the British Legation,* Peking.
HARDING, B. M., I Chow Fu.
HODGES, P. C., *Peking Union Medical College Hospital.*
HOWARD, H. J., *Department of Ophthalmology, Peking Union Medical College.*
HSIEH, E. T., *Department of Anatomy, Peking Union Medical College,* (*Councillor*).
INGLE, L. M., *Department of Anatomy, Shantung Christian University,* Tsinanfu, Shantung.
INGRAM, J. H., *American Board Mission,* Peking.
INOUYE, M., *Department of Anatomy, Tokyo Imperial University,* Tokyo, Japan.
LI DING, *Department of Anatomy, Hangchow Provincial Medical School,* Hangchow.
LI PAU CHEN, *Department of Anatomy, North China Union Medical School for Women,* Peking.
LIEU, T. C., *Hunan-Yale Medical School,* Changsha, Hunan.
MAIN, D. DUNCAN, Hangchow.
MAXWELL, J. P., *Peking Union Medical College.*
MEDHURST, C. SPURGEON, Peking.
MORSE, W. R., Changtu, West China.
MERRINS, E. M., *St. John's University,* Shanghai, (*Councillor*).
NEAL, J. B., *Shantung Christian University,* Tsinan, Shantung.
ONO, SHUNICHI KOISHIKAWA, Kobinatadai II, No. 35, Tokyo.
PACKARD, CHARLES, *Department of Biology, Peking Union Medical College,* (*Secretary-Treasurer*).
PHILLIPS, E. MARGARET, 13 Nan Wan Tzu Hutung, Peking.
PORTER, L. C., *Peking University,* Peking.
PORTERFIELD, W. W., *St. Johns University,* Shanghai.
RIDGE, W. S., Peking.
READ, BERNARD E., *Department of Physiological Chemistry, Peking Union Medical College.*
ROSENIUS, ELSA, Peking.
SCHUMAKER, ARTHUR, Tsung Pu Hutung, Peking.
SHIELDS, R. T., *Department of Histology and Embryology, Shantung Christian University,* Tsinanfu, Shantung, (*Councillor*).
SNELL, HOHN A., Soochow.
STEVENSON, P. H., Lu Chow Fu, Anhui.
STONE, R. S., *Department of Anatomy, Peking Union Medical College.*

TANG, E. H., *Director, National Medical College*, Peking. (*Councillor*).
TAYLOR, H. B., Anking.
TING, V. K., *Director, Geological Survey*, Peking, (*Councillor*).
VAN BUSKIRK, J. D., *Severance Union Medical College*, Seoul, Korea.
WILDER, J. W. D., Peking.
WILLIAMS, J. D., *Department of Anatomy, Hunan-Yale Medical School*, Changsha.
WU LIEN TEH, Peking.

CONSTITUTION

1. The Association shall be called the Anatomical and Anthropological Association of China.

2. The object of the Association shall be to advance the sciences of Anatomy and Anthropology in the Far East, in the broadest sense, especially in the coördination and centralization of activities, in the improvement of conditions for teaching and research and in the financing of special investigations and expeditions.

3. All persons who are interested in the objects of the Association shall be eligible for membership.

4. The names of candidates for membership shall be submitted to the Secretary-Treasurer at least one month before the meeting at which they come up for election.

5. Each member shall pay an initiation fee of $2.00 to the Secretary-Treasurer.

6. Annual dues shall be determined at the first annual meeting.

7. Members duly elected shall be able to assume life membership by the payment of $50.00, it being understood that the Association does not assume any obligations with respect to publications.

8. Upon the recommendation of the Council persons who further the interests of the Association either financially or in other ways shall be elected patrons of the Association.

9. The officers of the Association shall consist of a President, elected annually; and a Secretary-Treasurer, and twelve councillors, elected to serve for a period of two years.

10. The Council shall meet at the call of the President, in the interval between annual meetings for the consideration of expenditures, of recommendations for membership, and for the arrangement of programmes for meetings. The Council shall also deal with all other matters of concern to the Association and make recommendations to it for decision.

11. Annual meetings of the Association shall be held in close affiliation with the China Medical Missionary Association.

12. Special Meetings shall be held at the discretion of the President and at least three members of the council.

13. For the election of members, changes in constitution, extraordinary expenditures, and dropping of members, a three-fourths vote of those present will be required; for all matters of minor import, a simple majority vote will be sufficient.

PAPERS PRESENTED AT THE MEETING

1. *Relations of Anthropology to Medicine.* Aleš Hrdlička, U. S. National Museum, Washington, D. C.
2. *The Attitude of the Chinese Government towards Dissection.* S. P. Chen, Official Representative of the Board of Interior.
3. *The Jewish Colony at Keifengfu.* Dr. C. D. Tenney, American Legation, Peking.
4. *Stone Implements of Neolithic Type in China.* J. G. Andersson, Peking.
5. *Head Flattening.* E. T. C. Werner, Peking.
6. *Anthropologisches Studium Ueber Unterextremitaten der Chinesen.* Kotaro Shiino, Department of Anatomy, South Manchuria Railway Medical School, Mukden. (Read by title.)
7. *Observations made at the Second Chinese Government Animal Experimental Station.* Chou Wei-lien, Director.
8. *Methods of Anthropometry.* Aleš Hrdlička, U. S. National Museum, Washington, D. C.
9. *Ueber die Panethschen Zellen des Duodenums von Schweinen.* E. H. Tang, Director, Government Medical School, Peking.
10. *A Review of Chinese Anatomy from the Period of Huangti (Yellow Emperor 2697 B. C.).* E. T. Hsieh, Department of Anatomy, Peking Union Medical College.
11. *Seal Characters with Special Reference to Anatomical Terms.* J. H. Ingram, Peking.
12. *The Skull Measurements of Three Hundred Chinese.* S. H. Chuan, Director, Army Medical College, Peking.
13. *Height, Weight and Chest Measurements of 860 Chinese Students.* S. H. Chuan, Director, Army Medical College, Peking.
14. *Height, Weight and Chest Measurements of Healthy Chinese.* A. C. Hutcheson, Nanking.
15. *A Comparative Study of Physical Measurements of Chinese Students.* Arthur Schumaker, Peking. (Read by title.)
16. *The Origin of the Vitreous.* Harvey J. Howard, Peking Union Medical College, Peking.
17. *The Secretion of Urine in the Camel.* B. E. Read, Peking Union Medical College, Peking.
18. *The Application of X-Ray Study to Certain Anatomical Problems.* Paul C. Hodges, Peking Union Medical College. (Read by title.)
19. *A Report on the Wistar Institute of Anatomy and Biology and its Relation to Biology in China.* Chi Ping, The Wistar Institute, Philadelphia, Pa., U. S. A. (Read by title.)
20. *The Comparative Anatomy of the Mastoid Region.* Jui Hua Liu, Peking Union Medical College.
21. *The Endocranial Anatomy of Oreodon (preliminary report),* Davidson Black, Department of Anatomy, Peking Union Medical College.
22. *The Birds of North China.* G. W. D. Wilder, Peking.

23. *The Innervation of the Soft Palate.* M. Inouye, Department of Anatomy, Tokyo Imperial University, Tokyo, Japan.
24. *The Relation of the Interstitial Cells of the Reproductive Organs to Secondary Sex Characters in the Domestic Chicken.* Alice M. Boring, Peking Union Medical College.
25. *A Study of the Differentiation of Blood Cells in the Bone Marrow with the Aid of Janus Green and Other Supravital Dyes.* E. V. Cowdry, Department of Anatomy, Peking Union Medical College. (Read by title.)
26. *Some Growth Chances in the Walls of the Thorax in the Fetus.* C. K. Roys, Department of Anatomy, Shantung Christian University, Tsinanfu. (Read by title.)
27. *The Surgical Anatomy of the Ovary with Special Reference to the Blood Supply.* S. J. Kirkby-Gomes, Peking. (Read by title.)
28. *Cytological Reinvestigations on the Somatic Cells of Ascaris with Special Reference to Mitochondria.* ScunIchi Ono, Tokyo.
29. *The Effect of Starvation and Refeeding upon the Mitochondria, the Golgi Apparatus, and other Cytoplasmic Constituents.* ShunIchi Ono, Tokyo.
30. *The Effect of Radium on Cell Division.* Charles Packard, Peking Union Medical College.
31. *The Present State of the Schistosome Problem.* E. C. Faust, Peking Union Medical College.
32. *Problems under Investigation in Connection with the Collection of Human Embryos.* E. V. Cowdry, Department of Anatomy, Peking Union Medical College. (Read by title.)

DEMONSTRATIONS

1. *The Innervation of the Muscles of the Soft Palate.* Michio Inouye, Department of Anatomy, Tokyo Imperial University.
2. *Preparations of Mitochondria in the Somatic Cells of Ascaris.* ShunIchi Ono, Tokyo.
3. *Preparations Illustrating the Effect of Starvation and Refeeding upon the Mitochondria, the Golgi Apparatus and Other Cytoplasmic Constituents.* ShunIchi Ono.
4. *Experimental Alterations on the Mitochondria of Plant Cells.* N. H. Cowdry, Department of Anatomy, Peking Union Medical College.
5. *Microscopial Preparations of the Interstitial Cells of Sebright Testes.* Alice M. Boring, Peking Union Medical College.
6. *A Demonstration of the Behavior of Mitochondria in Bone Marrow Cells by Supravital Staining with Janus Green.* E. V. Cowdry, Department of Anatomy, Peking Union Medical College.
7. *Schistosome Larvae in Man and Other Animals.* E. C. Faust, Peking Union Medical College.
8. *Methods of Anthropometry.* Aleš Hrdlička, U. S. National Museum, Washington, D. C.

9. *A Demonstration of Some of Professor Dogiel's Original Preparations of Nerve Endings.* ShunIchi Ono, Tokyo.
10. *Preparations Illustrating the Effect of Feeding Meat and Fat upon the Mitochondria in the Acinus Cells of the Pancreas of the Guinea Pig.* Ma Wen Chao, Department of Anatomy, Peking Union Medical College.

It is hoped that this Association will open up lines of investigation which were formerly closed to anatomists as individuals, and that it will be successful in enlisting the active coöperation of many workers who are not professional anatomists.

China is a veritable *terra incognita* which offers unique opportunities for pioneer work along certain lines. The establishment of the U. S. National Research Council, alone, is sure indication that science is passing from an individualistic to a coöperative basis. As Dr. Livingston aptly remarks: "It has been occasionally suggested that one of the reasons for the slow advance of science lies in the fact that scientific research problems are still generally attacked by individuals or by small, local groups of workers influenced by a single individual, rather than by planned coöperation among a number of workers in different institutions. Individualistic research has been characterized, by the late Professor C. E. Bessey, as a kind of guerilla warfare upon the unknown." The wonderful discoveries which were made during the war are due to the fact that in a national emergency individual plans and aspirations are abandoned and coördination becomes the order of the day.

In the Orient, racial problems are uppermost, several of which have been indicated in a recent address by Hrdlička (1920). Anatomists are certainly in a position to contribute valuable information relating to the physical standards and potentialities of the Chinese race, which, in the last analysis, must form the basis for the adjustment which is bound to take place between the East and the West. If an arrangement can be made whereby careful records are kept of all the dissections carried on in the principal medical schools for a period of five or six years, a number of interesting facts are sure to be brought to light. A tabulation of the frequency of the chief progressive and regressive variations will be helpful in forming an opinion as to whether the

Chinese are a progressive or regressive type, and will, perhaps, indicate what evolutionary tendencies they exhibit along certain lines. Realizing that a thorough knowledge of physical standards is also prerequisite to any concentrated programme for public health work in China the members of the China Medical Missionary Association, through their Research Committee, have made a special study of the height, weight and chest measurements of healthy Chinese, and further investigations along similar lines are contemplated.

Friendly coöperation is also necessary for the successful study of vertebrate palaeontology and in order to collect data bearing upon the ancestry of man. We are told (Matthew, 1915) that mankind differentiated in Central Asia and migrated from this region to all parts of the world, and there seems to be a strong probability that in the course of time "missing links" of great importance will be discovered in China. The Director of the Geological Survey of China appreciates the importance of this type of investigation and has indicated his willingness to coöperate, so that a well organized attempt is now being made to collect specimens and data. A movement has also been set on foot in Peking largely through the initiative of Dr. Hrdlička, for the establishment of a Natural History Museum. At present research work along several lines is greatly handicapped through lack of museum facilities. A few isolated collections occur here and there, made chiefly by private individuals. Those of Arthur Stanley and of Kurz in Shanghai, and of Gee in Soochow, are of special interest. Through the generosity of the Rockefeller Foundation our own laboratory is well equipped for comparative work.

An attempt is being made to stimulate research work in embryology through the collection of Chinese embryos and fetuses, and by offering facilities for their study. In less than a year 83 specimens have been collected and we have managed to arrange for the shipment of specimens from different parts of the country to Peking. While most of them come at present from hospitals under foreign control we are unremitting in our efforts, with the help of our students and graduates, to obtain them directly

from native physicians because this source, if it can be made available, should prove to be almost inexhaustible.

Thus far I have referred to the desirability of coöperation in the collection of specimens and data; the more difficult type of coöperation, in actual laboratory experimentation directed toward the solution of definite anatomical and biological problems, though very desirable, will probably not be realized in China for many years to come on account of the dearth of persons trained in experimental work.

BIBLIOGRAPHY

China Medical Board of the Rockefeller Foundation, First, Second, Third and Fourth Annual Reports, 1915, 1916, 1917 and 1918.

Council on Medical Education Reports. China Medical Journal, 1913, 1915, 1917, 1920.

Cowdry, E. V. 1919 Plea for the Formation of an Anatomical Association in China. China Medical Journal, Vol. 33, pp. 517.

Cowdry, E. V. 1919 An Appeal for Human Embryos. Privately printed in Peking.

Cowdry, E. V. 1920 Anatomy in Japan, Anatomical Record, Vol. 18, p. 67.

Fischer, W. 1915 Die deutsche Medizinschule für Chinesen in Shanghai. Münch. mediz. Wochenschr.

Hrdlčika, Aleš 1920 The Anthropology of Asiatic Peoples. China Medical Journal. vol. 34.

Hsieh, E. T. 1921 A Review of Ancient Chinese Anatomy. Anatomical Record, vol. 20. This paper will appear in Jan., 1921, p. 97.

Kleinweg de Zwaan, J. P. 1917 Völkerkundliches und Geschlichtliches über die Heilkunde der Chinesen und Japaner. Nat. verh. van d. Hollandsche Maatschappij d. Wetenschappen te Haarlem, Derde Verzameling, Zevende Teel, Haarlem, De Erven Loosjes, 656 pp.

Livingston, Burton E. 1920 Constructive Scientific Research by Coöperation. Science, vol. 51, p. 277.

Matthew, W. D. 1915 Climate and Evolution. Annals of the New York Academy of Sciences, vol. 24, p. 171.

Report on Medicine in China by the China Medical Commission of the Rockefeller Foundation, 1914, University of Chicago Press.

Resumen por el autor, Eugene L. Settles,
University of Missouri.

Los efectos de una dieta grasa abundante sobre el crecimiento del tejido linfoide.

Dos gatos jóvenes procedentes de la misma cría fueron alimentados durante cuatro meses y medio, uno con leche que contenía 6 por ciento de grasa, el otro con la misma cantidad de leche con 3 y medio por ciento de grasa, suplementada en el caso del primero por carne grasa y en el último por carne magra. Despues del primer mes ambos presentaban excelente salud. El gato que recibía alimento rico en grasa presentaba un exceso en el peso del cuerpo y órganos, como indican las siguientes cifras: Peso total (despues de deducir el exceso de grasa), 30%; timo, 84.8%; glandula linfática mesentérica, 52.7%; bazo, 23.8%; hígado, 93.7%; páncreas, 80.6%; tibia izquierda, 44.1%; fémur izquierdo, 34.6%; corazón, 21.1% y riñones, 27 por ciento.

De estas cifras se deduce que el timo y la glándula linfática mesentérica, junto con el hígado y páncreas, presentan un exceso de peso relativamente mucho mayor que el exceso del total. El estudio microscópico demostró un exceso aun mayor en la cantidad de tejido linfoide a lo largo del canal alimenticio (tónsilas palatinas y faríngeas, placas de Peyer y folículos solitarios). Las placas de Peyer eran tres veces mas grandes que lo normal y se hallaron numerosos folículos solitarios y acumulaciones linfoides mas pequeñas en el gato alimentado con la dieta mas rica en grasa, mientras que en el otro no se pudo hallar ningun folículo solitario. De estos datos el autor concluye que el tejido linfoide responde con marcadas diferencias en su cantidad a las diferencias en la dieta, consistentes en diferencias en el contenido de grasa y valor en calorias. Estos dos factores no han podido separarse aún.

Translation by José F. Nonidez
Cornell Medical College, New York

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED BY
THE BIBLIOGRAPHIC SERVICE, NOVEMBER 1

THE EFFECT OF HIGH FAT DIET UPON THE GROWTH OF LYMPHOID TISSUE

EUGENE L. SETTLES

Anatomical Laboratory of the University of Missouri

SIXTEEN FIGURES (3 PLATES)

In spite of the universal interest in and the innumerable studies which have been made upon lymphoid tissue, very little has been learned regarding the factors which regulate its normal growth. It is known that lymphoid tissue is relatively much larger in amount during infancy and childhood and that it diminishes during adult life. It is also known that enlargement of lymphoid tissue may result from the effects of acute or chronic inflammation. However, the great variations in the lymphoid tissue, which are well known to occur, especially during childhood, form an unsolved problem.

Since the most important differences in the factors to which young animals are subjected are those associated with differences in diet, it seemed worth while to investigate the effect of varying diets on the amount of lymphoid tissue. The food element which seemed most likely to have a specific effect was thought to be fat, and for the following reasons.

Schäfer ('85, '12), who has made numerous studies on the relation between leucocytes and fat absorption, concludes from his own work and that of others that leucocytes, some varieties of which are formed in lymphoid organs, are known to take up fat in the villi and transport it to the lacteals.

Kischensky ('02), in studying the intestinal epithelium of young kittens during fat absorption, found leucocytes containing fat. From his description we may conclude that "these small round cells with large nuclei" are lymphocytes. He also found fat droplets in the macrophages of the lymph glands four to six hours after a meal.

E. R. and E. L. Clark ('17) observed that leucocytes are attracted to and actively ingest injected fat in the transparent tails of living amphibian larvae. Again, lymphocytes contain lipolytic ferment, while polymorphonuclears contain proteolytic ferment (Fiessinger and Marie, '09).

Czerny ('07) says that, with hypertrophy of fat in children, he finds enlargement of the tonsils, thymus, adenoids, and of the deep and superficial lymph glands, and that with regulation of diet in adiposity, he finds a diminution in size of lymphatic organs.

Hutinel (according to Steehman, '10) says that in acute intestinal inflammation after healing of the intestinal processes themselves there is a persistent emaciation of the child due to the disturbance of function of the mesenteric lymph glands. He also studied mesenteric lymph glands of dogs in different stages of digestion, and concluded that during digestion fat is emulsified in the mesenteric lymph glands and further worked over, and that here there must be a real chemical change. He says that the colorless content found in lymph vessels is soap. He also says that all the lymph glands play a similar rôle in inanition.

Poulain ('02) states that lymph glands play a double rôle in digestion, in that there occurs in lymph glands both splitting and synthesis of fat. He also says that during infection there is a lessened lipase activity of lymph glands and concludes that this may be the explanation of the general disturbance of nutrition which is found in intestinal infection.

Steehman ('10) investigated the question of relationship of lymph glands to fat digestion, and his conclusion is that the lymph glands are definitely concerned in the digestion of fat, and he states that they are assimilative organs for the fat derived from the tissues.

Bartel and Stein ('06) quote Paltauf, who holds that the enlargement of the thymus in status lymphaticus is not the cause of death, but merely a symptom of the general disturbance of metabolism further characterized by enlargement of the tonsils, lymph glands, and other lymphoid organs. He also describes fatty livers in many of these cases.

According to Kanthack and Hardy ('94), a meal causes a considerable rise in the number of lymphocytes in the blood. In rabbits, after a meal, they may form from 70 to 80 per cent of leucocytes, while in man two hours after a full meal they form about 30 per cent of the white blood-cells. These authors conclude that active digestion produces lymphocytosis, while starvation decreases the number of lymphocytes.

Thus, through the literature runs the strain of evidence that the lymphoid tissue plays an important part in the metabolism of fat.

It is well known that increase in the specific functions of tissues results in an increase in size. Hence, if lymphoid cells are concerned with absorption, transportation, and possibly with digestion of fat, one might expect the organs producing them to increase in size with an increase in the amount of fat handled by them.

As noted in the review of literature, the immediate effect of fat feeding has been investigated, and it has been found that the number of lymphocytes in the blood stream increases markedly during digestion, but shows a decided decrease in number during inanition. No definite reference, however, was found in the literature to any studies on the effect of a long-continued high fat diet on the size of lymphoid organs. It was therefore decided to plan experiments to test this question.

In addition to the general physiological and anatomical interest of this question, there are possible clinical applications of great importance. If lymphoid tissue is regulated to any considerable extent by the amount of fat given in the food, it follows that some of the enlargement of tonsils and adenoids in children may be due to a too high fat diet, particularly in those predisposed to this trouble by heredity.

The experiments reported here were begun with the idea of discovering the effect of an abnormal increase or decrease in the amount of fat in the diet upon the growth of lymphoid tissue. Milk was selected as the basis of the diet because of the ease of handling its fat content as well as for its general nutritional qualities. Kittens were chosen as suitable animals for the experi-

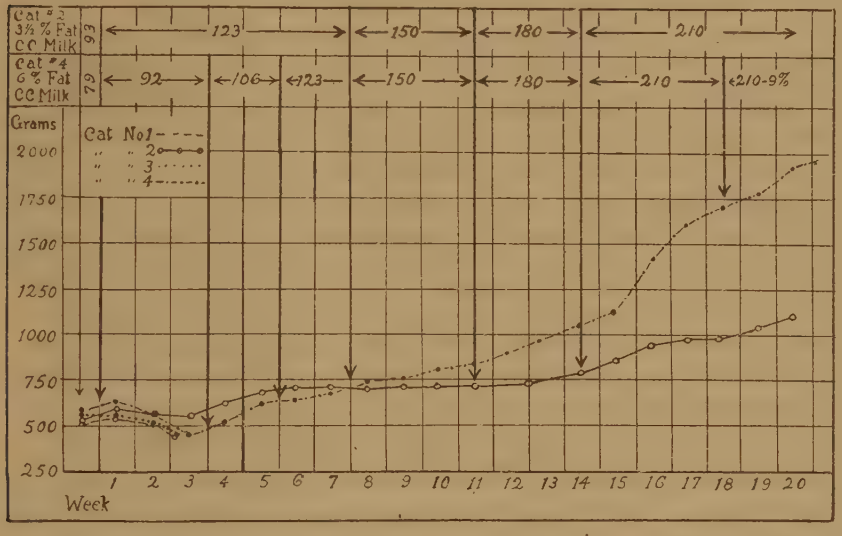
ment because of the possibility of feeding them at regular intervals and of controlling accurately the amount of food consumed at each meal. A litter of four kittens was obtained for the experiment. They were healthy animals, about three weeks old, and had not yet been weaned.

It was realized from the start that, if dependable results were to be secured, the animals would have to be attended to with greatest care, particularly as regards cleanliness of food, dishes, and cages, regularity of feeding, and mental condition. On account of the uncertainty of delegating such matters to a changing janitor force, it was decided from the start to feed and care for the animals personally. This was carried out from the beginning to the end of the experiment, except for short intervals when dependable substitutes were secured. The milk used was secured on alternate days from a reliable dairy and kept in clean bottles. During the warm weather the milk was kept on ice. The dishes were washed in hot water after each feeding. The floor of the cages consisted of wooden slats, the sides of fine-meshed wire. Water and a pan of sand were kept in each cage continuously, the sand being changed two or three times a week and the cages kept clean. Particular care was taken to keep the kittens free from all infections, parasites, or uncleanly conditions because of the possible effect of such agents upon lymph glands.

The kittens were fed the full amount of milk planned for them every day. At first they were fed three times a day, later twice a day, with an occasional day on which the total day's feeding was given at one time. The four kittens were kept in separate cages, with ample room for moving about. Throughout the entire experiment they were turned out of their cages frequently and allowed to play and exercise for an hour or more. They were treated kindly; in fact, they grew to be real pets.

Since no data were available as to the proper caloric requirement of young kittens, it was first necessary to determine the normal amount of milk consumed daily. A four-day test showed that the kittens consumed approximately 94 cc. of $3\frac{1}{2}$ per cent milk per 510 gram body weight or about 100 calories per kilo of body weight.

It was decided to feed the cats milk varying in fat content, as follows: no. 1, low fat, low calorie; no. 2, normal fat, normal calorie; no. 3, high fat, high calorie; no. 4, high fat, normal calorie. Based on the rough estimate mentioned above of 100 calories per kilo, the normal number of calories, and using $3\frac{1}{2}$ per cent fat as normal, the four cats were started on the diet shown in chart 1. The calorie contents, not shown in the chart, were: no. 1, 37.08 cal.; no. 2, 58 cal.; no. 3, 78.83 cal.; no. 4, 58 cal. The weights of the cats were, respectively, 510, 510, 538, and 510 grams.



This general plan was followed with increase in quantity once, until the death of cats no. 1 and no. 3. Thereafter, cat no. 4 was shifted to a high calorie diet, that is, it was given a daily amount of 6 per cent milk equal to the amount of $3\frac{1}{2}$ per cent milk given to cat no. 2. This was done in order to have a decided difference in the amount of fat given to the surviving cats.

The data concerning weight changes, amounts of milk given, with dates at which changes in amounts were made, are shown so fully in chart 1, that it is unnecessary to repeat them in detail. However, certain supplementary comments should be made.

On account of the difficulty of keeping the cats healthy on a pure milk diet, it was decided at the end of a week to give each cat a small amount of meat in order to keep them in good condition. Cat no. 2 received lean meat exclusively, while cat no. 4 was given meat containing small amounts of fat. The feeding of meat was continued throughout the remainder of the experiment, at two- or three-day intervals. This has been omitted from the chart. Changes in quantity were made whenever a stationary weight or increased greediness of the cats made it advisable.

A glance at the chart shows that none of the cats responded well to the change incident to weaning and confinement in separate cages. Instead of the rapid increase in weight which all animals of this age should show, there was a three-weeks period during which the weight remained stationary, as in cat no. 2, or declined, as in cats no. 1, no. 3, and no. 4. At the end of this period cats 1 and 3, which had been respectively on the low and high calorie diet, died, while cat no. 4 was in a very weakened condition. Although cat no. 2 appeared to be healthier than cat no. 4, still it maintained practically the same weight as at the start.

It is probable that the death of cats 1 and 3 and the poor condition of cats 2 and 4 toward the second of the third week were due to the change from mother's to cow's milk, accentuated in cats 1 and 3 by the markedly abnormal fat percentages.

After this, however, the condition of both cats no. 2 and no. 4 improved and continued excellent throughout the remainder of the experiment. From the third to the seventh week cat no. 2 increased slowly in weight. From the seventh to the twelfth week its weight remained practically stationary, even though the quantity of milk was increased at two different times. From the twelfth week to the end of the experiment its weight increased steadily, though slowly.

After the end of the third week when cat no. 4 almost died, it apparently adjusted itself to the high fat diet and began to gain weight, until, at the eighth week, its weight exceeded that of cat no. 2. From this time on until the end of the experiment the rate of gain of cat no. 4 was much more rapid than of cat no. 2.

During the last two weeks of the experiment, an attempt was made to increase still further the amount of fat consumed by cat no. 4, consequently 9 per cent milk was given during this period. However, during the first few days following this final change in diet, the cat did not drink the full quantity of milk. During the last week it accustomed itself to this extremely high fat percentage and at the time when the animals were killed it was consuming the entire amount of milk offered.

Cat no. 4 was killed on February 11th, nineteen weeks and three days after the beginning of the experiment. Cat no. 2 was killed two days later, at the same time of day as no. 4 and at exactly the same interval of time after the last feeding. The autopsies will be given later.

The history of the experiment, as represented graphically in the chart, shows that the original experiment of feeding four kittens of the same litter had be to modified owing to the death of two of the cats at a period before any of the four had become adjusted to cow's milk and confinement. Cat no. 2 received the same diet throughout, as regards fat percentage, and the amount of diet was determined by the natural appetite of the animal. While its growth was much slower than normal so that the total weight and size of organs cannot be considered normal, nevertheless it serves as a satisfactory control as regards the effect of differences in fat content of the diet. Cat no. 4, starting out with a diet of 6 per cent milk containing the same number of calories as the control animal, after the death of cat no. 3, was given the same quantity of 6 per cent milk as that of the $3\frac{1}{2}$ per cent milk fed to cat no. 2, hence it received a greater number of calories. During the latter half of the experiment this cat received some fat meat while cat no. 2 received nothing but lean meat. During the last few days of life, cat no. 4 was consuming the amount of 9 per cent milk equivalent to the quantity of $3\frac{1}{2}$ per cent milk taken by the control. Hence cat no. 4 was fed a high fat diet throughout the experiment, and after the fifth week it received a high calorie as well as a high fat diet.

The cats, as has been stated, were kept clean and free from parasites throughout the experiment, with the exception of a

small number of fleas which appeared at times and were treated with insect powder. The general condition of both was excellent during the last fifteen weeks.

AUTOPSIES

Cats no. 1 and no. 3

Owing to the fact that cats nos. 1 and 3 had failed to adapt themselves to cow's milk and had been in an increasingly weakened condition for several days before they died, it was thought that little would be gained from extensive study of the lymphoid organs. Careful autopsies showed nothing markedly abnormal in the thoracic and abdominal organs. The large mesenteric lymph gland from each cat was weighed and examined microscopically. The weight of the gland from cat no. 3, 1.470 grams, exceeded that from cat no. 1, 1.423 grams, by 0.047 gram. Microscopic examination, however, showed a definitely larger number of lymphoid cells making up the cortex and medullary cords and filling the sinuses in cat no. 3 as compared with cat no. 1.

Cats no. 2 and no. 4

As previously mentioned, both these cats were in perfect health at the end of the experiment; their coats of fur were smooth and silky and they were active and playful. Cat no. 4, which had been fed the high fat diet, was larger and heavier and conspicuously fatter than cat no. 2, which had received a normal percentage of fat. The two cats were killed at exactly the same hour of the day and at the same length of time after the last feeding.

Autopsy of cat no. 4 (male). Cat was killed with chloroform February 11th at 9:50 A.M. The last feeding was given at 2:30 P.M., February 10th. There is a layer of subcutaneous fat over the abdomen, about one-quarter of an inch thick. Much fat is present in the omentum. The peritoneal, pleural, and pericardial surfaces are smooth and shiny, with no increase in fluids in these cavities. The lacteals are well injected with fat. The

chief mesenteric lymph gland is homogeneous in appearance and seems large and swollen. It weighs 3.040 grams. The other small, scattered mesenteric lymph glands were not noticeably increased in number. The stomach and intestines show nothing abnormal from the outside. On opening, the stomach is found to contain a considerable amount of partly digested material and the small intestine much yellowish semisolid material. No worms or other parasites were visible to the naked eye in stomach or intestine. The mucous membranes showed no especial reddening or other abnormality.

In the lower part of the ileum, the Peyer's patches (folliculi agminati) stand out with exceptional clearness. They form mottled raised areas projecting into the lumen of the bowel clearly marked out from the surrounding mucosa. The largest patch is the one in the terminal ileum, just above the ileocecal valve, which measures 3 cm. in length. In the 12 cm. of intestine anterior to this there are four round patches, each about 1 cm. in diameter, all conspicuously raised over the surrounding surface. The pancreas is large and normal in appearance, weighing 9.055 grams. The spleen is dark red with numerous white spots, and has rounded edges. It weighs 3.060 grams. The liver is very large with rounded edges, and has a yellowish color. It weighs 118 grams. The right and left kidneys are dark red in color, showing nothing abnormal. Their weights are 6.5 and 6.7 grams, respectively. The lungs are air containing and normal, save for one small atelectatic area in the upper right lobe. The heart is surrounded by a considerable amount of fat. It shows nothing abnormal. Its weight is 7.9 grams. One each of the right bronchial and right inferior cervical lymph glands were examined and preserved. They show nothing unusual macroscopically. The thymus is homogeneous in appearance and seems unusually large. It weighs 5.495 grams. The right and left tonsils are covered with hemorrhagic spots. In order to preserve them without injury for histological study, some of the surrounding tissue was dissected out with them, therefore it was not possible to estimate their weight accurately. The pharyngeal tonsil which is located at the top and posterior wall of the pharynx, is

easily seen as a definitely raised area containing many raised whitish translucent spots. The left femur was preserved. It weighs 6.557 grams, while its greatest length is 7.8 mm. The left tibia was also preserved. Its weight is 5.200 grams and the greatest length is 83 mm. Most of the subcutaneous fat was dissected out. It weighs 109.4 grams. Either all or parts of the various organs were preserved in Zenker's fluid for microscopic study.

Autopsy of cat no. 2 (male). Cat was killed with chloroform, February 13th at 9:20 A.M. The last feeding was given at 2:30 P.M., February 12th. There is little fat present over the abdomen. The omentum likewise contains little fat. There is no increase in the fluid in the pleural, pericardial, or peritoneal cavities. Linings of all these cavities are smooth and shiny. The lymphatics are filled with chyle. The chief mesenteric lymph gland is homogenous in appearance. Its weight is 1.990 grams. The stomach and intestine show nothing abnormal externally. On opening, the stomach is found to contain a small amount of nearly digested food and the small intestine contains very little except some brown mucus. No worms or parasites were visible to the naked eyes in the stomach or intestine. The mucous membranes are apparently normal. On opening the lower end of the ileum, it is found to be practically empty. A long Peyer's patch is found here. It is scarcely raised above the general epithelial surface and its extent is difficult to determine. The remainder of the small intestine was searched carefully for other Peyer's patches, but none could be seen in the gross specimen. The presence of the brown mucus here possibly prevented us from discovering the smaller patches at the time; however, the entire small intestine was preserved for further examination. The pancreas shows nothing abnormal. It weighs 5.540 grams. The spleen has sharp, definite edges and its surface is mottled with small round nodules. It weighs 2.470 grams. The liver is normal apparently; it is dark red in color and has sharp edges. Its weight is 60.9 grams. The right and left kidneys are normal in appearance, weighing 5.1 and 5.3 grams, respectively. The right lung shows a small atelectatic area, otherwise both lungs are

normal. The heart is normal, weighing 6.5 grams. The thymus is very pale. It weighs 2.973 grams. The largest right inferior cervical lymph gland shows nothing abnormal macroscopically. It is very pale in color. At the roof of the pharynx in the place where the pharyngeal tonsil was easily seen in cat no. 4, only a faint mottling can be seen. On dissecting this tissue loose from the bone and examining it carefully, definite small nodules may be seen. The left femur was preserved. It weighed 4.870 grams and its greatest length was 67.5 mm. The left tibia was also preserved. Its weight is 3.607 grams and its greatest length 73 mm.

DISCUSSION OF AUTOPSY FINDINGS OF CATS NO. 2 AND NO. 4

The postmortem examination confirmed the observations of the living animals that the two animals were entirely free from disease. It is noteworthy that no parasites were found.

The difference in the weight of these two animals at the time of death has already been referred to. It was obvious merely from the superficial inspection at the time of autopsy that the subcutaneous fat, the fat in the omentum and around the heart was noticeably greater in the cat which had received the high fat diet. Moreover, the macroscopic appearance of the liver, with its rounded edges and yellow color, made it appear highly probable that the difference in the weight of this organ in the two cats was due to an extra fat content in cat no. 4. (This was later confirmed by histological findings.) Aside from these differences in stored fat, the most noticeable difference in the two cats was found in the lymphoid tissue. Even without the comparison of the weights of the different lymphoid organs, the greater size of those in the fatter cat was very apparent. This was epecially noticeable in the case of the Peyer's patches. In the cat fed on high fat diet, the Peyer's patches were large, discrete, conspicuous, and prominently raised above the intestinal wall, while those of the cat fed on normal fat percentages were scarcely raised above the epithelial surface and so inconspicuous as to make their detection very difficult from macroscopic examination. The same contrast, in somewhat less degree, was noticeable in the case of the pharyngeal tonsils. The lymphoid tissue

in the pharynx of cat no. 4 was fairly thick and well raised above the surface, while in this same region in cat no. 2 the lymphoid tissue was represented merely by a faint mottling of the roof of the pharynx.

The comparison of a number of the organs of the two cats is best brought out by a chart showing the weights. These weights were all obtained in the fresh state.

Chart 2 gives the weights of the larger lymphoid organs (such as mesenteric lymph glands, thymus, spleen, etc.) of the two cats and also the weight of other organs (such as the heart, kidney, liver, etc.) for the sake of comparison. The smaller lymphoid organs, such as the Peyer's patches, the palatine tonsils, cervical lymph glands, and pharyngeal tonsils, could not be accurately weighed on account of the necessity of leaving some of the surrounding tissue attached to them, hence the comparison of these was based upon microscopic study.

In chart 2, the weights of the two cats and the absolute and relative weights of their various organs are first given. The excess body weight of cat no. 4 over cat no. 2 is next shown, together with the excess weight of its various organs.

For several reasons the body weight of cat no. 4 should be corrected, in order to obtain a fair comparison of the various organs. It has been mentioned that 109.5 grams of subcutaneous fat was found in cat no. 4, while cat no. 2 had very little subcutaneous fat. Again, the liver of cat no. 4 proved to contain an enormous amount of stored up fat. Beside these two factors, the gastro-intestinal contents were much greater in cat no. 4 than in cat no. 2. Now it would seem that the organs which would give the best relative comparison between animals such as these two cats at the end of the experiment would be the heart, kidneys, and perhaps the skeleton. On obtaining the average of the percentages by which cat no. 4 exceeds cat no. 2 with respect to heart, kidneys, tibia, and femur, the result arrived at is approximately 30 per cent. This same figure is obtained approximately if the excess subcutaneous fat (109.5 grams) plus a portion of the liver weight (37.5 grams), which accounts for only part of the stored-up fat, be subtracted from the gross body weight

CHART 2

Absolute and relative weights of body and organs, cat no. 2 and cat no. 4

ALL WEIGHTS GIVEN IN GRAMS	TOTAL BODY	THYMUS	SPLEEN	MESENT. LYMPH GLAND	LIVER	PANCREAS	LEFT TIBIA	LEFT FEMUR	HEART	RIGHT KIDNEY	LEFT KIDNEY
Cat no. 2 (3½ per cent fat), absolute weight....	1219	2.973	2.470	1.990	60.9	5.540	3.607	4.870	6.50	5.10	5.30
Cat no. 2, per cent of body weight.....		0.24	0.2	0.16	5.0	0.454	0.296	0.40	0.533	0.418	0.434
Cat no. 4 (6 per cent fat), absolute weight.....	1729	5.495	3.060	3.040	118.0	9.055	5.20	6.557	7.9	6.5	6.7
Cat no. 4, corrected weight.....	1582										
Cat no. 4, per cent of corrected body weight.....		0.35	0.19	0.19	7.46	0.572	0.329	0.414	0.50	0.41	0.423
Excess weight, cat no. 4 over cat no. 2.....	363[1]	2.522	0.592	1.050	57.1	4.515	1.593	1.687	1.4	1.4	1.4
Per cent excess, cat no. 4 over cat no. 2.....	30[1]	84.8	23.8	52.7	93.7	80.6	44.1	34.6	21.1	27.4	26.4
Difference between excess per cent of organs and excess per cent of corrected body weight.....		+54.8	—6.2	+22.7	+63.7	+50.6	+14.1	+4.6	—8.9	—2.6	—3.6

[1] For these figures the corrected weight of cat no. 4 is used.

of cat no. 4. Thus, subtracting 147 from 1729, the resultant figure 1582 is obtained as the corrected weight of cat no. 4, which is 363 grams, or 30 per cent in excess of the weight of cat no. 2. Were the excess gastric and intestinal contents also subtracted, the figure would probably be lowered to about 25 per cent, which is about the average by which the heart and kidneys of no. 4 exceed no. 2. However, for safety, the figure 30 per cent is chosen for the table.

With this new percentage excess as a basis, the percentage excess of the various organs of cat no. 4 over cat no. 2 are calculated. By this means a truer estimate is arrived at of the actual effect of the difference in diet on the individual organs.

It is seen from the corrected percentage table at the bottom of chart 2 that the organs may be divided into three groups:–1) those apparently affected by the difference in diet, i.e., the liver, the thymus, pancreas, and mesenteric lymph gland; 2) those which may have been affected by the difference in diet, i.e., femur and tibia, and, 3) those which were apparently not affected by the difference in diet, i.e., the kidneys, spleen, and heart. Discussion is reserved until the microscopic findings have been given.

MICROSCOPIC STUDY OF ORGANS FROM CATS NO. 2 AND NO. 4

The various organs were fixed in Zenker's fluid for the most part. They were dehydrated in graded alcohols and aniline oil, cleared in xylol, imbedded in paraffin, and sectioned at 10 μ or 15 μ. Since this technique proved to be unsatisfactory in the case of the thymus, portions of this organ were imbedded in celloidon. Bouin's fixative and the method of dehydration described by Allen ('19) were also tried, but the tissues treated in this way proved to be too hard to section well. The sections were stained with Mayer's haematoxylin and eosin. The attempt was made, in each case, to secure specimens and sections from the two cats which corresponded as exactly as possible, as regards the part of the organ from which they were taken, the plane of sections, and the technique employed.

The chief object of the microscopic study was to find out the difference in the amount of lymphoid tissue in the lymphoid organs of the two cats. Measurements with the micrometer eyepiece were made of the sections of the different lymphoid organs of the two cats. However, the wide variation in the shape of these organs in the two cats and the uneven distribution of the lymph follicles and cords as well as the varying sizes and distribution of vessels, connective tissue, and spaces in the organs of the two cats make this method rather unreliable. The most satisfactory comparisons were derived from observation of the sections and from microphotographs taken at the same magnification.

Liver. The liver of cat no. 2 (control) is apparently normal histologically. In contrast to this, the liver of cat no. 4 (fed on the high fat diet) is packed with fat globules of various sizes to such an extent that the whole liver structure is altered and its regularity destroyed. The cell structure is also changed by reason of the stored fat. The liver cells present a spongy appearance due to the presence in them of fat globules of various sizes. In many places the fat globules are so large that it is difficult to determine whether they are inside the liver cells or whether they are intercellular.

The microscopic findings in the liver of cat no. 4 adequately explain the differences noted in the gross specimens of the two cats, especially the immense difference in the weight of the two organs.

Pancreas. No significant histological difference was found in the case of this gland from the two cats.

The microscopic study of the lymphoid organs will now be considered.

Cervical lymph gland. The variability in shape and size of lymph glands makes comparison of the smaller glands unsatisfactory. Fortunately, the cat possesses an enormous mesenteric lymph gland, on which comparisons of size are feasible. The cervical glands, one from each cat, are selected as samples of smaller glands and are to be compared merely as regards relative distribution of lymphoid tissue, as seen with the microscope.

While the cortex of the gland from cat no. 2 is wider at one point than the cortex of that of cat no. 4 at its widest point, a glance at the figures of the two glands (figs. 1 and 2) shows that this gives an erroneous idea of the comparative amount of cortical substance present in the two glands. In cat no. 4 the cortex maintains a fairly even thickness throughout the entire circumference—a thickness which in no place falls far below the maximum. In cat no. 2 the cortical substance is either missing entirely or very narrow over approximately two-thirds of the circumference, being present only as two separated masses at the two ends of the section. Recognizable follicles are present in both glands. They are more numerous in the gland from cat no. 4, in which they are arranged fairly regularly around the circumference of the gland, with an occasional follicle more deeply placed. In the medulla of cat no. 2 the cords are narrow and somewhat irregular, but in cat no. 4 they are much wider and more continuous. The sinuses contain many more lymphocytes in the gland from cat no. 4 than in that from cat no. 2.

It is clear, from the comparison of the glands, that there is a definitely greater amount of lymphoid tissue in the gland of cat no. 4 than in that of cat no. 2.

Pharyngeal tonsils. As may be seen in figure 4, a cross-section of the pharyngeal tonsil of cat no. 2, the lymphoid cells are somewhat sparsely distributed in the reticulum. Two definite large solitary follicles are present. The lymphoid cells are more closely packed around the edges of these follicles than at any other place in the section. In the centers of the follicles the lymphoid cells are somewhat grouped together, leaving definite clear spaces between.

In the pharyngeal tonsil from cat no. 4, figure 3, the lymphoid cells are much more closely packed together. The solitary follicles are crowded with lymphoid cells. There is a much larger amount of lymphoid tissue outside the follicles than in the gland of cat no. 2, and the lymphoid cells here are more closely packed together.

The measurements of the lymphoid tissue of a typical cross-section of the pharyngeal tonsils are as follows:

Cat no. 2, greatest length, 3.5 mm.; greatest width, 0.52 mm.

Cat no. 4, greatest length, 5 mm.; greatest width, 1 mm.

The approximate areas of the cross-sections are: Cat no. 2, 1.82 sq.mm., and cat no. 4, 5 sq.mm. Since the sections are through comparable parts of the glands and since care was taken to secure exact cross-sections, these figures give a rough estimate of the relative volumes of the two glands, namely, a proportion of 1 to 2.7. In view of the relatively greater density of the lymphoid cells in the gland from cat no. 4, it is probable that the pharyngeal tonsil from cat no. 4 contains in the neighborhood of three times as much lymphoid tissue as that from cat no. 2.

Palatine tonsils. A comparison of these is somewhat unsatisfactory, owing to the difficulty of orienting them for obtaining corresponding sections. However, since their oval shape makes possible a cross-section perpendicular to the long axis, and by selecting the sections through the largest part of each gland, a fair comparison is secured. Two such sections, pictured in figures 5 and 6, show the following.

The section from cat no. 2 (fig. 6), is oval in shape. There is a narrow open space in the middle, which represents the crypt which extends deep into the gland.

Twelve solitary follicles may be counted arranged in a single marginal layer. The periphery of the follicles as well as the spaces between follicles are densely packed with lymphoid cells. Bordering the crypt, lymphoid cells are more densely packed than in the surrounding area.

In the section from cat no. 4 (fig. 5) the crypt is shown extending to the surface. There are twice as many follicles—twenty-four—as in the gland from cat no. 2, and they occur in two and three layers. The epithelium lining the crypt is invaded and, in many instances, largely replaced by lymphoid cells, which form a dense area near the lumen. The distribution of lymphoid cells is about the same as in cat no. 2.

Measurements of sections of the two glands are as follows:

Cat no. 2, greatest length, 3.4 mm.; greatest thickness, 1.8 mm.

Cat no. 4, greatest length, 5.4 mm., greatest thickness, 2.5 mm.

The areas of the two sections are in approximately the ratio of one to two for the sections from cats no. 2 and no. 4, respectively.

Although these are only rough approximations of the size of the gland, all the data point to the gland from cat no. 4 as being very decidedly—probably at least 100 per cent—larger than the gland from cat no. 2, which, with a similar relative distribution of lymphoid cells, means a decidedly greater total amount of lymphoid cells in the cat receiving the higher fat diet.

Spleen. The sections shown in figures 9 and 10 are taken from the widest portions of each gland. In the section from cat no. 2 (fig. 8) a considerable number of red blood-cells may be seen scattered among the lymphocytes and other cells. The lymphoid cells are numerous and evenly distributed. The malpighian corpuscles are rather small and contain definite germinal centers. The section from cat no. 4, figure 7, contains a greater number of red blood-cells. The malpighian corpuscles are more numerous than in cat no. 2 and definitely larger. The germinal centers have about the same appearance as in cat no. 2, possibly not as distinct.

It will be remembered that there was very little difference in the weight of the two spleens, considering the difference in the size and weight of the two animals. The microscopic study, however, shows a slight difference in the lymphoid tissue in the two cats. The spleen of cat no. 4 contains perhaps slightly more lymphoid tissue than that of cat no. 2, due to the increased number and definitely larger size of the malpighian corpuscles. However, the difference is hardly enough to justify the conclusion that the spleen has been modified by the difference in diet.

Thymus. Figures 9 and 10 show the thymus gland of cat no. 4, and no. 2, respectively. These typical sections are very difficult to compare microscopically, owing to the great difference in the shape and arrangement of the lobules. Moreover, a comparison of the relative size of the medulla and cortex in the lobules is inaccurate as the section goes through the different lobules at different levels.

In the section shown from cat no. 2 some of the lobules show a definite demarcation between cortex and medulla, while others show none, according to the part of the lobule cut through in the

section. The cortex of the lobules is densely packed with lymphoid cells, the medulla being comparatively free from them.

In the section of thymus from cat no. 4 there are clearly more large lobules present than in cat no. 2. Whether the total number is greater is very difficult to say, owing to the irregularities in size and shape. There is little difference in the relation of cortex to medulla in cat no. 4 as compared with cat no. 2. The place in the lobule through which one section passes affects this relationship, as in cat no. 2.

Apparently, then, the most important point in the microscopic comparison of the thymus of cat no. 2 and the thymus of cat no. 4 is the larger size of lobules found in the latter. Since the total weight shows a percentage excess for the thymus from cat no. 4 of 54.8, as shown in chart 2, and since the microscopic study shows lymphoid tissue to form at least an equal percentage of the entire gland, there is an excess percentage of lymphoid tissue of at least 50.

Mesenteric lymph glands. The sections were taken through the enlarged part of the glands, at which place they were of about the same diameter. Both glands at this point contain two definite lobes, separated by connective tissue.

Figures 11 and 12 show sections of the two larger lobes of cat no. 4 and no. 2, respectively. These two lobes are about the same size. In the section from cat no. 4 we notice a decidedly great number of solitary follicles which are also larger and show a thicker and denser rim of lymphoid cells, as compared with the gland from cat no. 2. In cat no. 4 these follicles are found in the medulla as well as in the cortex. Few are seen in the medulla of the gland of cat no. 2. The cortex of cat no. 4 apparently contains more lymphoid tissue than that of cat no. 2, but the difference is not great. However, the most striking thing about the sections is the comparison of the medulla. There is in cat no. 4 a greater area of medulla than in cat no. 2. The cords in the medulla are greater in number and decidedly larger and more continuous. Likewise, the sinuses cover a greater area and contain a great many more lymphocytes in the gland from cat no. 4 than are found in cat no. 2, where the sinuses are comparatively

free from lymphocytes. The two smaller lobes of cat no. 2 and cat no. 4 compare in practically the same way as the larger lobes.

The comparison of these two typical sections, then, shows but little difference in the comparative area occupied by lymphoid cells in the two glands. The medullary cords are larger and the follicles are larger and more densely packed with cells at the periphery in cat no. 4 than in cat no. 2. However, the extrafollicular accumulations of lymphoid tissue are perhaps greater in cat no. 2. Other sections of these glands were studied and showed similar conditions. Since, however, the gland from cat no. 4 showed a percentage weight which was 22.7 per cent higher than that from cat no. 2, it is obvious that the percentage amount of lymphoid tissue present in the mesenteric lymph gland of cat no. 4 exceeds that of cat no. 2 by at least 22.7 per cent.

Peyer's patches (*agminated follicles*). By far the most striking difference found in any of the lymphoid organs was that found in the comparison of the Peyer's patches, as seen through the microscope. Cross-sections of the long patch located in the terminal portion of the ileum are shown in figures 13 and 14 and parts of them shown again at a higher magnification in figures 15 and 16.

A comparison of the dimensions of the cross-sections is as follows:

Width of patch, cat no. 4, 7.5 mm.; cat no. 2, 5.2 mm. Average thickness of patch, cat no. 4, 2.2 mm.; cat no. 2, 1.04 mm.

Since this patch has been found in several cats to be of approximately the same length and of fairly uniform width, it is possible to estimate, from the two dimensions given, the relative volumes of the Peyer's patches in cats no. 2 and no. 4. The areas of the cross-section are found to be: for cat no. 4, 16.5 sq.mm.; for cat no. 2, 5.4 sq. mm. It follows that the volume of the gland from cat no. 4 is three times the volume of the gland from cat no. 2. This is a far greater difference than has been found in any of the organs in which comparison by weight was possible. Moreover, a study of the sections shows an equally striking difference in the histological picture. The section from cat no. 2 shows a row of nine follicles, lying mainly between the muscularis mucosae

and the circular muscle layer, with a fairly wide stretch of submucosa intervening between the follicles and the latter. Toward the lumen, these follicles have projections, most of which end at the muscularis mucosae. A few of them break through this layer and extend out into the base of the villi, forming wedge-shaped structures. Between the follicles there is some lymphoid tissue in the half of the patch toward the lumen, while in the other half there is loose tissue. The villi over the patch are, in the main, normal appearing, save for the few whose bases are invaded by the wedge-shaped lymphoid projections, though perhaps they are slightly shorter than over the remainder of the mucosa.

The section from cat no. 4 shows extraordinary differences. There is about an equal number of follicles, but the individual follicles are enormously larger—in fact, as seen in figures 15 and 16, they may well be called gigantic—as compared with those from cat no. 2. The space at the submucosa between the follicles and the circular muscle layer is somewhat less than in no. 2. The follicles are packed with lymphoid cells. Toward the lumen, the muscularis mucosae is broken through at many more places than in no. 2, and the wedge-shaped projections into the villi are much larger and more numerous. The villi over the patch are very short and wide, many containing a central mass of lymphoid tissue. The spaces between adjacent follicles are smaller than in no. 2, and are filled with lymphoid tissue in the half toward the lumen, as in cat no. 2.

It is obvious that the proportion 1 to 3, found for the volumes of the patches, probably underestimates the relative amounts of lymphoid tissue, since the follicles are equally packed, while in no. 4, the projections through the submucosa are much greater and the spaces between the follicles much less.

A very suggestive finding, also, was that of the presence on cat no. 4 of isolated follicles, separated from the Peyer's patch, usually one or two in each section. None were found in cat no. 2. This suggested that new follicles may have developed in cat no. 4 and led to a study of the lower ileum to determine how extensive this difference might be.

Solitary follicles and lymphoid accumulations in the lower ileum. In order to obtain an estimate of the relative number and size of solitary follicles in the lower ileum, five blocks of intestinal tissue from cat no. 4 and six from cat no. 2 were examined. The pieces, ½ to 1 cm. long, were taken at various corresponding levels, from the lower third of the small intestine. The blocks were embedded in paraffin and cut transversely into sections 25 μ thick. Every fifth section was saved. The results are as follows:

Cat no. 4: block (1) 38½ cm. above caecum in	35 sections	6	solitary follicles
Cat no. 4: block (2) 25½ cm. above caecum in	29 sections	19	or lymphoid
Cat no. 4: block (3) 20½ cm. above caecum in	25 sections	17	accumulations
Cat no. 4: block (4) 16 cm. above caecum in	45 sections	35	
Cat no. 4: block (5) 11 cm. above caecum in	42 sections	61	
Total	176 sections	138	
Cat no. 2: No. (1) 39 cm. above caecum in	63 sections	0	solitary follicles
Cat no. 2: No. (1a) 32 cm. above caecum in	46 sections	0	or lymphoid
Cat no. 2: No. (2) 25 cm. above caecum in	56 sections	0	accumulations
Cat no. 2: No. (3) 20½ cm. above caecum in	55 sections	0	
Cat no. 2: No. (4) 15 cm. above caecum in	50 sections	0	
Cat no. 2: No. (5) 10 cm. above caecum in	54 sections	0	
Total	324 sections	0	

In estimating the number of follicles in cat no. 4, follicles appearing in more than once section were counted once only. Peyer's patches seen in some of the blocks from both specimens were disregarded.

The 'follicles' seen in cat no. 4 vary from small accumulations of lymphoid cells, located outside the muscularis mucosae, to full-sized follicles, many of which project through the muscularis mucosae into the base of the villi.

The result of this estimation is most striking. In the intestine of cat no. 2, 324 sections were examined, and no trace was found of lymphoid accumulations or solitary follicles, apart from Peyer's patches. In cat no. 4, in only a little more than half as many sections, 138 separate lymphoid accumulations or solitary follicles were seen, in addition to Peyer's patches. This result is the most striking one which has been obtained and harmonizes with the study of the two Peyer's patches. There can be no question

but that the difference in diet is an exceedingly important factor in the regulation of the amount of lymphoid tissue in the intestinal wall.

DISCUSSION AND CONCLUSION

In reviewing the results of the experiments, it should be emphasized that whatever effect may have been produced by the difference in diet cannot be attributed unequivocally to the mere difference in the fat content of the diet, since there was also a difference in the amount of calories. The experiment, therefore, shows the effect of a high calorie diet, in which the excess calories are provided by the fat of cow's milk. With this definitely understood, let us group together the results which have been obtained.

A comparison of the weights of organs has shown that there has been produced, in the animal receiving the richer diet, an enlargement of pancreas, liver, thymus, and mesenteric lymph gland out of proportion to the enlargement of other organs, such as heart and kidneys. Gross appearance and the study of microscopic sections have also shown a striking enlargement of the lymphoid tissue along the digestive tract—of the palatine and pharyngeal tonsils—and especially of the Peyer's patches and solitary follicles. Microscopic study and comparison of the elements making up the lymphoid organs showed the lymphoid tissue to be either equally extensive or more extensive, relatively, in the cat fed the high calorie, high fat diet as compared with the control. The excess weight and size were found to be due not to stored-up fat (except in the liver), but to increase in lymphoid tissue proper. Therefore, it is obvious that, in lymph gland and thymus, with a decided excess in weight, and at least an equal distribution of lymphoid tissue, the high calorie, high fat diet has resulted in a very substantial increase in the amount of lymphoid tissue proper. In the two tonsils and particularly in the Peyer's patches, all of which are made up of practically solid lymphoid tissue, the difference in size as seen in corresponding sections—and which amounts, in the case of the Peyer's

patches, to a 200 per cent excess—indicates a decidedly larger relative amount of lymphoid tissue as a result of the higher diet.

An exception is furnished by the spleen, which though a lymphoid organ, shows no excess weight nor any marked increase in lymphoid tissue proper. It might be urged, in explanation, that the spleen is extremely variable in size in normal animals, as has been pointed out by Mivart ('98) and Hatai ('15), among others, and that a larger number of experiments might yield a result different from the present single experiment. However, this would be unjustifiable, considering the similarity of treatment of the two animals. It is only fair to conclude that the size of the spleen and the amount of lymphoid tissue contained in it have not been affected by the difference in diet.

The very great excess increase in the thymus is of extreme interest. It has been found by many observers that the thymus is remarkably sensitive to undernourishment. Its response is so striking that Hammar ('05) has given the name 'accidental hunger involution' to the great reduction in size which occurs. Jonson ('09) found in rabbits, after four weeks of underfeeding, a reduction in weight of thymus to one-thirtieth the weight of the control. Jackson ('15) observed a loss of 90 per cent relative weight in the thymus of rats given a maintenance diet between the ages of three and ten weeks. Stewart ('18) obtained, in rats held at maintenance diet from birth to ten weeks, an 80 per cent loss in relative weight of the thymus. Jonson ('09) found an extremely rapid recovery rate on resumption of normal feeding, normal weight being reached after three weeks of normal diet following a period of undernourishment.

The present study indicates that the thymus responds to a surplus diet, in which the surplus calories are supplied by fat, by a decided increase in size. It thus supplements and supports previous studies which shows the thymus to be particularly sensitive to differences in diet.

The similarity in response of the thymus and of the lymph glands and lymphoid tissue along the intestinal tract affords evidence in support of the theory that the thymus should be grouped with lymphatic glands as a lymphoid organ.

The results furnish new evidence in support of the view, suggested at the beginning of the paper in the review of literature, that the lymphoid organs are concerned with metabolism—probably with fat metabolism—for they show a marked response to the high fat diet, along with the liver and pancreas, which are known to be specifically concerned with the storage and digestion of fat. In responding to an increased function, or demand, for lymphoid cells, or lymphocytes, by an increase in size, the lymphoid organs would be merely following the well-proved law of functional adaptation, according to which increase in the function of an organ is accompanied by increase in size.

Further studies should be made in order to analyze the two factors of high calorie and high fat, which have been combined in the present experiment, and also to test the effect on lymphoid organs of other dietary factors. However, whether the results have been caused by one or the other, or both, they are of value, for they show that these organs respond very decidedly to differences in diet, by differences in size, which are far out of proportion to the relative differences in the size of the entire animals.

SUMMARY

Four kittens of the same litter and practically the same weight were fed on diets differing in fat content, with the purpose of discovering the effect of a high fat diet on lymphoid tissue.

Two of the animals, one of which had been fed on a low fat percentage (0.7 per cent) and the other a high fat percentage (6 per cent), died at the end of three weeks. No appreciable difference was found in the gross appearance or weight of the lymphoid tissue of these two cats. However, microscopic examination showed a noticeably greater amount of lymphoid tissue in the mesenteric lymph gland of the cat which had received the high fat diet.

The other two kittens were kept for a period of four and one-half months, one on a normal fat percentage diet ($3\frac{1}{2}$ per cent) and the other on a high fat percentage diet (6 per cent). They both remained in perfect health throughout the last three and a half months of this time.

Although the two cats started with the same size and body weight, at the end of the four and a half months the cat fed on higher fat diet weighed 510 grams more than the control, and was practically 30 per cent larger, excluding the amount of stored fat present.

In the cat fed on a high fat percentage diet, the organs known to be concerned with fat metabolism and storage (the pancreas and liver) were noticeably larger, both by actual weight and percentage than in the control specimen.

In addition, the lymphoid tissue, with the exception of the spleen, was noticeably heavier in the cat receiving the high fat diet, the excess amounting to a difference of 85.8 per cent in the case of the thymus and 52.7 per cent in the case of the mesenteric lymph gland.

In contrast to this, the heart and kidneys were only about 25 per cent larger in the larger animal.

Microscopic examination of similar cross-sections from all the lymphoid organs of the two animals showed either an equal or a greater amount of lymphoid tissue per unit area in the cat which had been fed on the high fat diet. The difference was most strikingly shown in the case of the Peyer's patch and the solitary follicles in the intestine.

It is pointed out that the results obtained indicate that a combined high fat, high calorie diet produces a general enlargement of the lymphoid tissue of the body, which is most strikingly seen in the lymphoid tissue of the gastro-intestinal tract, but that they do not differentiate between the two factors—high calorie and high fat. An analysis of these two factors as well as the effect of other food elements should form the subject of further investigations.

My sincerest thanks are due Dr. E. R. Clark for his invaluable advice and interest in the problem here presented.

BIBLIOGRAPHY

ALLEN, EZRA 1919 A technique which preserves the normal cytological conditions in germinal and interstitial tissue in the testis of the Albino rat. Anat Rec., vol. 16, no. 1, p. 25.

BARTEL AND STEIN 1906 Über abnormale Lymphdrüsen befunde und deren Beziehung zum Status thymico-lymphaticus. Archiv. f. Anat. und Physiol., Anat. Abt., S. 231.

CLARK, E. R. AND E. L. 1917 A study of the reaction of lymphatic endothelium and of leucocytes in the tadpole's tail, toward injected fat. Am. Jour. Anat., vol. 21, p. 421.

CZERNEY 1907 Zur Kentniss der exudativen Diathese. Monatschr. f. Kinderh., Bd. 6, S. 1.

FIESSINGER AND MARIE 1909 Les ferments des leucocytes dans les exudats des séreuses. C. R. de l. Soc. de Biol., p. 1062; also, Jour. de Physiol., T. 11, p. 746.

HAMMAR 1905 Zur Histogenese und Involution der Thymusdrüse. Anat. Anz., Bd. 27, S. 41.

HATAI 1915 The growth of the body and organs in Albino rats fed with a lipoid free ration. Anat. Rec., vol. 9, p. 5.

JACKSON, C. M. 1915 Changes in the relative weights of the various parts, systems and organs of young Albino rats held at constant body weight by underfeeding for various periods. Jour. Exp. Zool., vol. 19, p. 135.

JENSON, AVID 1909 Studien über die Thymusinvolution: Die Akzidentelle Involution bei Hunger. Arch. f. Mikr. Anat., Bd. 73, S. 390.

KANTHACK AND HARDY 1894 The morphology and distribution of the wandering cells of mammals. Jour. Physiol., vol. 7, p. 81.

KISCHENSKY, D. 1902 Zur Frage über die Fettresorption im Darmrohr und den Transport des Fettes in andere Organe. Ziegler's Beitr., Bd. 32, S. 197.

MIVART, ST. GEORGE 1898 The cat, p. 181. New York: Chas. Scribner's Sons.

NEWTON, R. C. 1919 A case of enlarged thymus gland and some remarks on status lymphaticus. Amer. Jour. of Med. Sciences, vol. 167, p. 354.

POULAIN 1902 De l'action des ganglions lymphatiques sur l'absorption et la resorption des graisses. Revue mens. des Maladies de l'enfance, T. 20, p. 289.

SCHÄFER, E. A. 1885 On the part played by amoeboid cells in the process of intestinal absorption. Int. Monat. für Anat. und Physiol., Bd. 2, S. 1.
1912 Textbook of microscopic anatomy, pp. 396, 405, 521, 548, 551, 676, 681. New York: Longmans, Green & Co.

STHEEMAN, H. A. 1910 Histologische Untersuchung über die Beziehungen des Fettes zu den Lymphdrüsen. Beitr. z. Path. Anat. U. Z. allg. Path. Jena, Bd. 48, S. 170–204.

STEWART, C. A. 1918 Changes in the relative weights of the various parts, systems and organs of young albino rats under-fed for various periods. Jour. of Exp. Zool., vol. 25, p. 333.

EXPLANATION OF FIGURES

Figures 1 to 16 are microphotographs of histological sections of organs from cats no. 2 and no. 4. They are arranged in pairs—in each case the even numbers (2, 4, 6, etc.) placed at the right, being taken from the organs of cat no. 2, the odd numbers (1, 3, 5, etc.) at the left, from cat no. 4. The two pictures shown in each pair were taken at exactly the same magnification. The enlargement varies for the different pairs. The two pairs shown in figures 15 and 16 show, at a higher magnification, parts of the same sections as are shown in figures 13 and 14, respectively.

PLATE 1

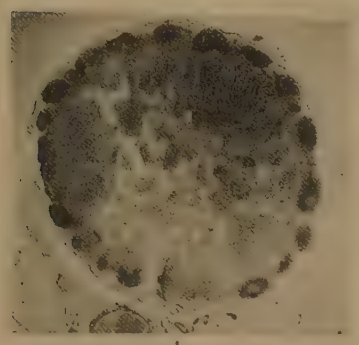
EXPLANATION OF FIGURES

1 and 2 Cross-sections of inferior cervical lymph glands, 10 μ thick. Figure 1, cat no. 4; figure 2, cat no. 2.

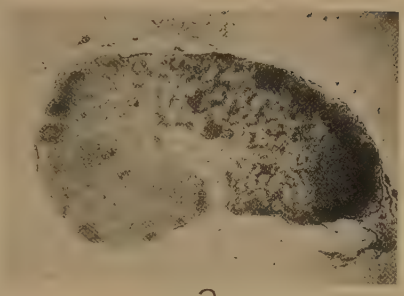
3 and 4 Cross-sections of pharyngeal tonsils, 10 μ thick. Figure 3, cat no. 4; figure 4, cat no. 2.

5 and 6 Cross-sections of right palatine tonsils, 10 μ thick. Figure 5, cat no. 4; figure 6, cat no. 2.

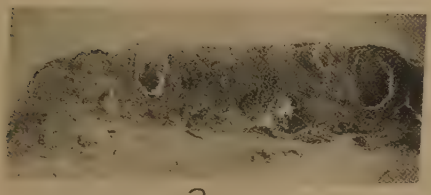
7 and 8 Cross-sections of spleen, taken at the widest parts of the organs, 10 μ thick. Figure 7, cat no. 4; figure 8, cat no. 2.



1



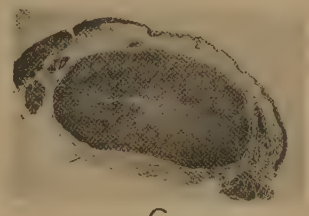
2



3

4

5



6

7

8

PLATE 2

EXPLANATION OF FIGURES

9 and 10 Cross-sections of corresponding parts of thymus; embedded in celloidin, and cut 15 μ thick. Figure 9, cat no. 4; figure 10, cat no. 2.

11 and 12 Cross-sections of mesenteric lymph glands, 10 μ thick. Figure 11, cat no. 4; figure 12, cat no. 2.

13 and 14 Cross-sections of the long Peyer's patch in the terminal ileum, 20 μ thick; magnified 4.25 (approx.). Figure 13, cat no. 4; figure 14, cat no. 2.

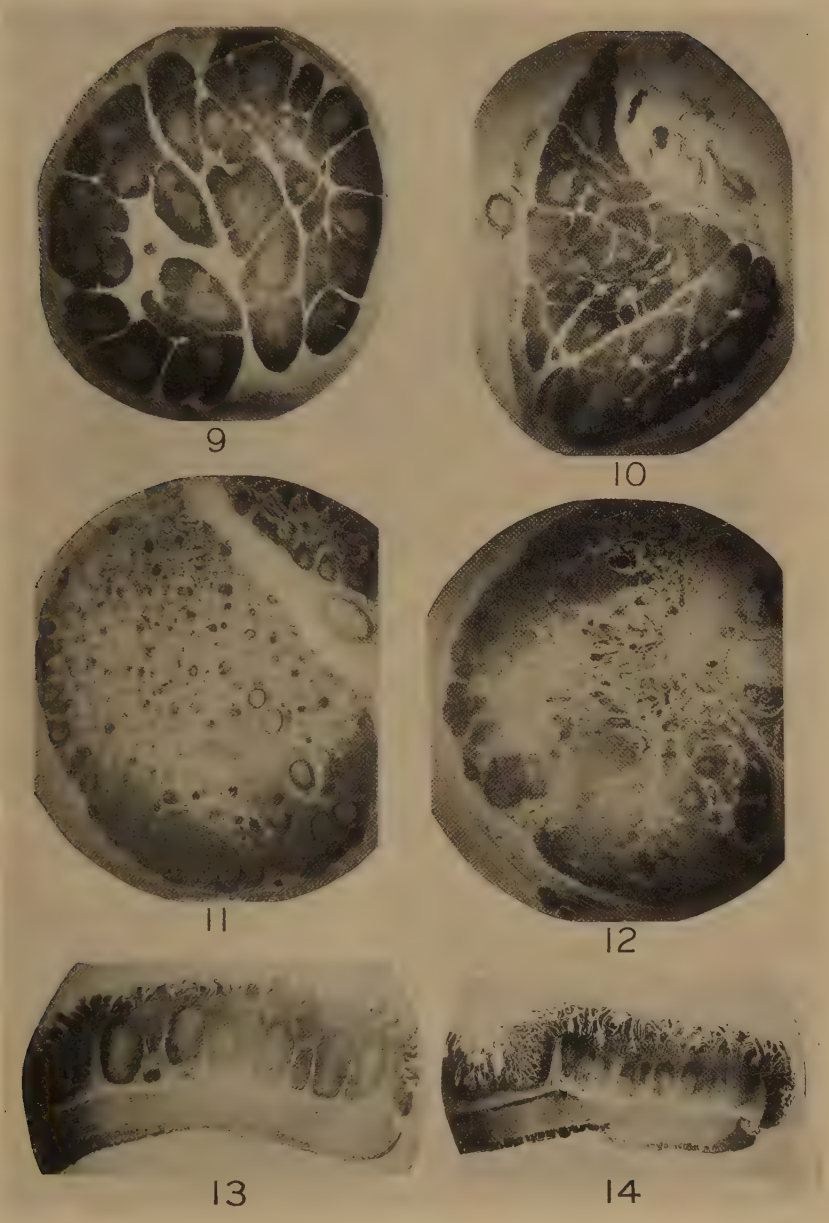
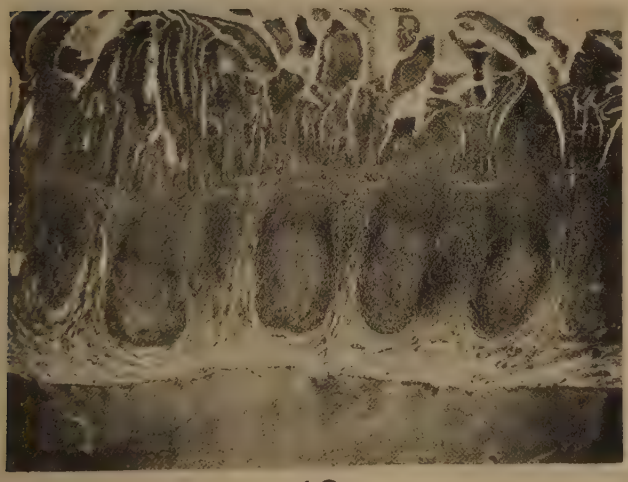

9
10
11
12
13
14

PLATE 3

EXPLANATION OF FIGURES

15 and 16 Higher-power magnification of parts of same sections as shown in figures 13 and 14.; magnified 18 times (approx.), Figure 15, cat, no. 4; figure 16, cat no. 2.

15



16

Resumen por el autor, Ivan E. Wallin,
University of Colorado.

Un caso de persistencia de la vena supracardinal izquierda con dos venas espermáticas izquierdas.

La descripción de este caso consiste principalmente en una ilustración de las variaciones venosas del sujeto estudiado. La porción inferior de la vena cava inferior consta de dos troncos. La vena espermática izquierda esta representada por dos vasos distintos; uno de ellos comunica con la vena renal y el otro con la vena supracardinal izquierda, que persiste en este caso.

Translation by José F. Nonidez
Cornell Medical College, New York

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, NOVEMBER 15

A CASE OF PERSISTENT LEFT SUPRACARDINAL VEIN WITH TWO LEFT SPERMATIC VEINS

IVAN E. WALLIN

Department of Anatomy and the Henry S. Dennison Research Laboratories, University of Colorado

ONE FIGURE

The occurrence of duplication of the inferior vena cava is not an altogether rare condition. In recent years Givens[1] has described two cases observed in the dissecting room of Cornell University Medical College, Ithaca. He also reviewed the literature of some fourteen cases.

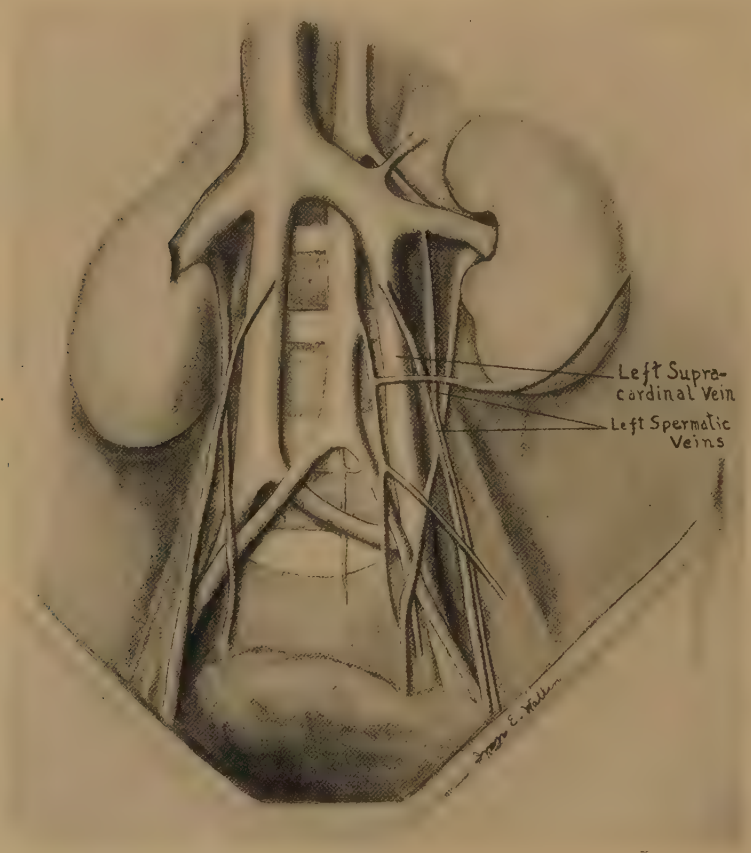
The venous variant here recorded was found in a male cadaver in the dissecting room at the University of Colorado School of Medicine. There were no records of the age or the cause of death. The approximate age was sixty-five years.

Figure 1 illustrates the lower part of the caval system in this subject. In character it is quite similar to the veins found in subject no. 466 described by Givens. The left persistent supracardinal is larger than in Givens' subject. In his specimen the size ratio between the right and left supracardinals was 3 to 1.6. In my subject the ratio is about 2 to 1.5.

An unusual feature in this subject are the two left spermatic veins. So far as I can learn this is the first recorded instance of such a condition. The figure shows the nature of the two veins. The one emptying into the renal vein was about one-third the size of the one emptying into the left supracardinal. The two veins were dissected out for some distance in the cord. With the exception of their proximal parts where they separate to empty into the above-named veins they lie close together in all of their course.

[1] Givens, M. H., 1912. Duplication of the inferior vena cava in man. Anat. Record, 6: 475–486.

The condition found by Huntington and McClure[2] in a human subject may help to explain the duplication of the left spermatic vein. They found fenestrae in the renal collar which established connections between the right spermatic vein and the right renal vein. By a further extension of the fenestrae along the vein a condition might be produced which would be like the character of the veins in this subject.



[2] Personal communication from Professor McClure.

THE ANATOMICAL RECORD, VOL. 20, NO. 2,
JANUARY, 1921

Resumen por E. V. Cowdry, por el autor E. T. Hsieh,
Peking Union Medical College of Rockefeller Foundation.

Una revisión de la antigua anatomía china.

Basándose en un conocimiento íntimo de los clásicos chinos, el autor presenta una revisión de la historia de la Anatomía en China comenzando con el gran "Emperador Amarillo," Huangti (2697 A. J.). Primero revela el notable conocimiento de esta ciencia por Huangti y luego demuestra que el trabajo llevado a cabo ulteriormente ha sido dominado por sus enseñanzas. El autor deduce que Huangti y algunos de sus sucesores practicaron la disección humana y que este método directo de observación "fué prontamente reemplazado por un dominio de autoridad algo semejante al que reinó en Europa antes del Renacimiento."

Translation by José F. Nonidez
Cornell Medical College, New York

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, DECEMBER 6

A REVIEW OF ANCIENT CHINESE ANATOMY

E. T. HSIEH

Anatomical Laboratory of the Peking Union Medical College of the Rockefeller Foundation

THIRTY-ONE FIGURES

INTRODUCTION

Chinese civilization is probably as old or even older than that of Egypt, but unfortunately we have no written records to cover the periods of the Emperor of Heaven, the Emperor of Earth, and the Emperor of Man, each of which is supposed to have covered ten thousand years. According to traditions which have been handed down to us, the earliest Chinese built their homes in trees, clothed themselves in the furs of animals and drank their blood. They kept records by making knots on strings, but it is not until the time of the three Emperors (2852 B.C. to 2697 B.C.[1]) and the Five Rulers (2697 B.C. to 2255 B.C.) that history may be said to begin. The subsequent literature contains many interesting references to anatomy, and Dr. E. V. Cowdry has asked and encouraged me to review it from this point of view. I am particularly indebted to the Peking Union Medical College for purchasing the necessary books, some of which are rare and expensive.

Before entering upon a detailed discussion of the gradual development of anatomy in China, it will be necessary for me to describe briefly the nature of the written record and to mention in review the chief Chinese medical classics.

We read that Tai-Hao (1) and his servant Fei Lung (2) are to be credited with making the first attempt at recording words by painting diagrams as far back as 2852 B.C.

[1] All the dates expressed in terms of A.D. and B.C. are taken from E. T. C. Williams; Dictionary.

Chinese writing, however, really dates from the time of the 'Yellow Emperor,' Huang-ti (3), 2697 B.C., who ordered his servant Tsang Chih (4) to make words in the form of imitative symbols. These were done in lacquer upon strips of bamboo or palm leaves. Paper and pens came much later. It is said that the vertical arrangement of characters was adopted because it is much easier to write up and down a bamboo than around it. Many characters were elaborated during this period. We are told that Shen-Nung saw a good crop and made a character representing crop ears; that Huang-ti wrote a 'cloud;' Yao (5) a 'turtle;' Yü (6) the 'bell and the kettle,' and so on. The symbols devised represented pieces of string of different lengths with knots at various places in their course. Those consisting of a single knot with a small piece of string attached have suggested the name of 'tadpole characters' for this writing. These characters soon became profoundly modified.

Figures 1 and 2 illustrate the pictorial type of the writing of the Hsia dynasty, 2205 B.C.

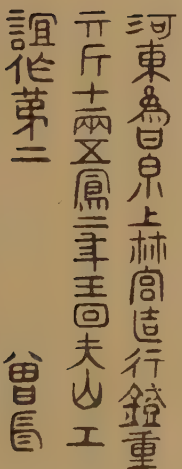
A considerable change was also made about the beginning of the Chin dynasty (7), 255 B.C., when Li Si (8) greatly improved the terms and divided them into Ta Chuan (9) and Hsiao Chuan (10). Hsiao Chuan was changed to Chin Chuan (11) during this period. A little later Cheng Miao (12) devised a script called Li Shu (13), or plain square writing, which the first Chin emperor observed and ordered to be used throughout his empire. Paper was first manufactured at this time by Wang Lun (14).

In the Han dynasty (15), 206 B.C., writing was further improved by Chia Fang (16), San Tsang (17), Tsai I (18), and Shih Ching (19), who wrote the word, Han Li (20), as may be seen by reference to figure 3. In the Chin (21) dynasty, 265 A.D., Chung Yin (22) and Wong Hsi Chih (23) changed the writing into a style more nearly approaching that of the present day. Typical Chi-dynasty (479 A.D.) writing is well illustrated in figure 4, and this may be compared with the modern Chinese on pages 125–127.

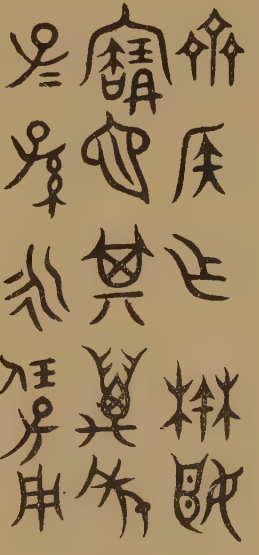
The works of Huang-ti (3), written over four thousand years ago, 2697 B.C., are the most interesting and original of the great

1

2



3



4

Fig. 1 Old style of writing, Hsia dynasty, 2205 B.C.
Fig. 2 Old style of writing, Hsia dynasty, 2205 B.C.
Fig. 3 Old style of writing, Han dynasty, 206 B.C.
Fig. 4 Old style of writing, Chi dynasty, 479 A.D.

Chinese medical classics. They stand at the basis of native Chinese medicine. They were written on bamboo strips in words of the tadpole style as a record of questions which Huang-ti asked his servant Chi Pe (24). Later, in the Han dynasty (206 B.C.), they were gathered together into book form and were called *Neiching* (25) *Internal Classics, Su Wen* (26), *Inquiries* and *Ling Shu Ching* (27), *The Classics of the Living Spirit.* Unfortunately, they were composed in such genuine literary style that they were difficult to understand until the time of Tang (28), in 620 A.D., when Wang Ping (29) wrote explanatory notes for each book.

In the Chow dynasty (30), 1122 B.C., there lived an anatomist called Chin Yueh Jen (31), who wrote a treatise about the viscera and arteries and called it *Nanching* (32), or *Hard Classics.* This volume contains, among other things, a number of interesting measurements of the weights of the different organs of the men of that time.

In the Han dynasty, 206 B.C., Chang Chi (33) wrote two books (34 and 35) on *The Essentials of the Gold Medicine Case* and on *Fevers,* which are very popular to this day. *The Prescription for Emergency* (36), by Ke Hung (37), appeared in the Chin period (38), 265 A.D. and, about two hundred years later, we meet with Chu Cheng's (39) copy of the *Testament* (40).

Many other authors follow, of whom we may mention Tsao Yuén Fang (41) in the Suei dynasty (42), 689 A.D.; Sun Si Miao (43) and Wang Show (44), in the Tang dynasty (45), 620 A.D.; and Wang Kun, Shen Kue, Chen Chi, Tang Chi, Liu Wen, Han Ti, Pang An Shih, Cheng Ho Chung, Tang Shen, Wang Chu, Hsu Su, Chen Ssu Wen, Hsia Te, Chang Kuo, Chen Tze Ming, Chen Yen, Li Hsun, Yen Yung Ho, and Yang Chih Ying (46–64) —all in Sung dynasty (65), 960 A.D.

Liu Wen Su, Chang Yuan Su, Chang Chung Cheng, and Li Hao (66–69) were prominent medical writers in the Chin (70) dynasty (1200 A.D.), and Wang Hao Ku, Sha-Tu-Ma-Su, Wei Ye Lin, Chu Chen Ting, Wang Kue Juei, Chi Te Chi, Tai Chi Tsung, and Wang Lü (71–78) in the Yuan dynasty (79), 1280 A.D.

The name of Chang Chi Ping (80) is famed as a great anatomist in the Ming dynasty (81), 1368 A.D. Chang wrote a number of books. His most famous one he called *Leiching* (82). It dealt intensively with the visceral and vascular systems.

Important advances were made in the Ching dynasty (83), 1644 A.D., when the Emperor Chien Lung (84) edited an *Encyclopedia of Chinese Medicine* (85) and the government encouraged research. Sheng Teng's (86) book on osteology (87) is replete with interesting observations. In the days of Chia Ching (88), 1796 A.D., a terrible epidemic raged among the children in the town of Chang Li Hsien (89) and many died. A certain magistrate, named Wang Chui Jen (90), visited the public cemetery and found that, since the children were buried in very shallow graves, the hungry dogs were able to uncover the bodies and devour them. Wang's curiosity was so stimulated that he went daily to the cemetery and observed over thirty complete bodies dismembered by the dogs. He was thus enabled to test out the old theories and to make important new observations which formed the basis for his book which he called *A Correction of Faults in Medicine* (91).

At present anatomy is out of date in native Chinese medicine because the doctors only prescribe herbs for the patients and make no attempt at surgical operations.

As it is impossible for me to attempt to review, in the space at my disposal, the whole of the ancient science of anatomy, I shall confine myself to a discussion of splanchnology, angiology, and anthropology.

HISTORY OF CHINESE SPLANCHNOLOGY

Splanchnology and angiology may be traced back to the time of Huang-ti, 2697 B.C. The two subjects were then more or less philosophically treated, and the theories advanced at that time have been handed down to us almost without change and constitute the foundation of native Chinese medicine to-day.

Life is said to depend upon the action of a female principle which embraces a male principle. These principles are opposite powers of vigor or strength which are equal in weight. When

they are properly balanced there will be no disease of any kind and the person will be productive and healthy. These principles are distributed quite differently in the body. The exterior is male and the interior female; the back male and the abdomen female; the viscera male and the parenchymatous organs are female. Each principle has three degrees in quality, namely, great female principle, female principle proper, and young female principle; great male principle, male principle proper, and young male principle (92–97). These three degrees of principle are evenly distributed in their respective organs and viscera.

There are twelve tracts for the transmission of these principles in the general circulation. Translating literally we read that: The hand receives the great female principle of the lung; the foot receives the great female principle of the spleen; the hand receives the female principle proper of the pericardium; the foot receives the female principle proper of the liver; the hand receives the young female principle of the heart; the foot receives the young female principle of the kidney. The hand receives the great male principle of the large intestine; the foot receives the great male principle of the bladder; the hand receives the male principle proper of the small intestine; the foot receives the male principle proper of the stomach; the hand receives the young male principle of the three burning spaces; the foot receives the young male principle of the gall-bladder.

The three burning spaces referred to are situated in the thorax, abdomen, and pelvis and are illustrated in figure 13. They are supposed to be filled with fatty tissue.

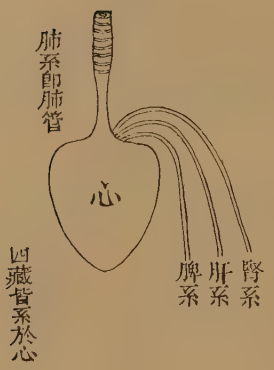
We read further, that, since the exterior is male and the interior female, then the male and female principles are as the coat and the lining (98). (Literally, great male and young female are the coat and the lining; male proper and young female are the coat and the lining; young male and great female are the coat and the lining.)

The quality of the principles varies in different organs and we have corresponding differences in the amount of air and of blood. The great male principle usually has much blood and very little air; the male principle proper has both blood and air

in good quantity; and the young male principle has very little blood but much air. The great female principle has much air and little blood; the female principle proper has much blood but little air; and the young female principle has very little blood but much air.

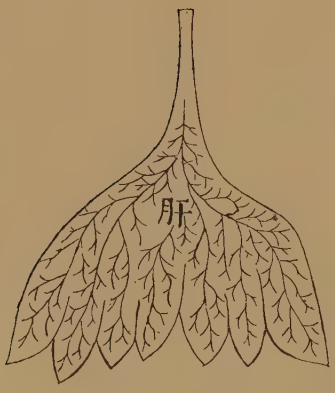
The liver (fig. 7), heart (fig. 5), spleen (fig. 8), lungs (fig. 9), and kidneys (fig. 16) are commonly called the five parenchymatous organs, while the gall-bladder (fig. 15), stomach (fig. 14), large intestine (fig. 16), small intestine (fig. 11), bladder (fig. 12), and the three burning spaces (fig. 13) are regarded as the six viscera. The pericardium (fig. 6) and brain may also be referred to as organs. There are some differences of opinion about this, however, because Chi Pe, the servant of Huangti, 2697 B.C., claimed that the brain, bone-marrow, gall-bladder, and uterus are premanent singular bodies, which would indicate that the brain and bone-marrow may also be classified as true organs. The meaning of the term organ (99) is to store up, but not to eliminate, while the word viscera (100) means to eliminate, but not to store up.

Huangti, 2697 B.C., describes in his book on *Neiching*, or *Internal Organs*, what the different organs store. We read that the liver (fig. 7) stores the blood, which contains the soul; that the heart (fig. 5) stores the pulse, which contains the spirit; that the spleen (fig. 8) stores the nutrition, which contains the thoughts; that the lungs (fig. 9) store the breath, which contains the energy, and, finally, that the kidneys (fig. 16) store the germinating principle, which contains the will. He explains also his idea of their action. The liver has a rancid odor, a sour taste, a brown color, makes the sound *chüeh* (101), a note in Chinese music, and at will is the seat of anger. The heart has an odor of toast, a bitter taste, a brownish-red color, makes the sound *chih* (102), and at will is the seat of happiness. The spleen has a fragrant odor, a sweet taste, a yellow color, makes the sound *kung* (103), and at will is the seat of thought. The lung has a fishy smell, a hot taste, a white color, makes the sound *sheng* (104), and is the seat of sorrow. The kidneys (fig. 16) have a putrid smell, a salty taste, a black color, make the sound *yu* (105), and at will are the seat of fright.



5

6



7

8

Fig. 5 Heart with three cords, as kidney, liver, and spleen (Huang-ti period, 2697 B.C.).
Fig. 6 Pericardium.
Fig. 7 Liver.
Fig. 8 Spleen.

These five organs are supposed to work harmoniously together and serve in the development of the body as follows: The liver produces the ligaments, forms the heart, and controls the lungs. The heart produces the blood, forms the spleen, and controls the kidneys. The spleen produces the flesh, forms the lungs, and controls the liver. The lungs produce the skin and hair, form the kidneys, and control the heart. The kidneys produce the bone-marrow, form the liver, and control the spleen.

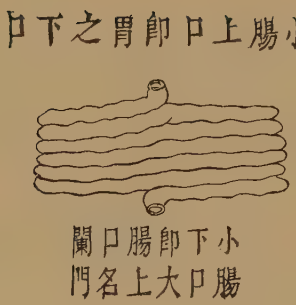
The five organs control the five senses and all parts of the body. The liver has an eye at its opening, converts the fluid into tears, supplies the ligaments, and nourishes the nails. The heart has the tongue at its opening, converts the fluid into perspiration, supplies the pulse, and nourishes the complexion. The spleen has the mouth at its opening, converts the fluid into saliva, supplies the flesh, and nourishes the lips. The lungs have the nose at their opening, convert the fluid into snivel, supply the skin, and nourish the fine hairs. The kidneys have the ears as their openings and also the genito-urinary region, they convert the fluid into spittle, supply the bone, and nourish the hairs.

The five organs are related to the six viscera which answer each other in their action (p. 103). The lungs relate to the large intestine, which answers the skin; the heart relates to the small intestine, which answers the arteries; the liver relates to the gall-bladder, which answers the ligaments; the spleen relates to the stomach, which answers the muscle, and, lastly, the kidneys relate to the three burning spaces and the bladder, which answer the skin and the hairs.

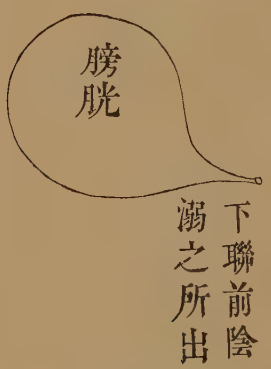
The functions of the organs and viscera are described as follows: The heart is the king who directs the body, the lungs are the promulgators who carry out his orders. The liver is the general whose duty it is to meditate carefully. The gall-bladder is the central legal officer, who makes judgments. The pericardium is the minister to bring happiness. The spleen is the officer of granaries who creates the five tastes. The large intestine is the officer of communications who starts all sorts of changes. The small intestine is the receiving office in which digestion is carried on. The kidney is the officer of vigor or strength who

9

10



11



12

Fig. 9 Lung with trachea (2697 B.C.).

Fig. 10 Large intestine with the upper opening for the small intestine and the lower part for the rectum.

Fig. 11 Small intestine with the upper and lower openings.

Fig. 12 Bladder and urethra.

serves through his intellect. The three burning spaces constitute the sewage system from which all the canals drain into the bladder where the fluid is stored; after having been acted upon by the air, it is finally passed out.

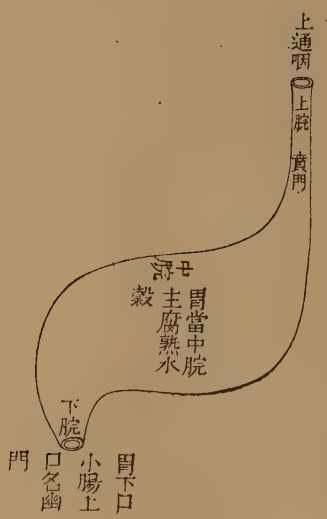
The surrounding walls of the organs and viscera are also described: The thorax and abdomen constitute the city wall and the pericardium (fig. 6) is the palace of the king; the stomach is the granary and the throat and small intestine the post-office. The five openings of the stomach are called the doors, entrances and outlets (of the granary). Water and grain enter the body by the esophagus and air by the trachea. The food enters the stomach where essence soaks into it and it becomes air, which, if of nourishing nature, passes into the lower burning space. The air, entering at the trachea, passes through the epiglottis, which is the door of the voice. The mouth and lips are the fan for the voice, the tongue the machine for the voice, and the uvula the pass for the voice. The larynx divides the air. The breath, coming from the lungs, is thought to act upon the transverse bone (hyoid) and the tongue (in speaking).

In the same book Huangti gives some measurements of the alimentary tract which will be of interest to anthropologists (see also p. 121).[2]

Distance from lip to teeth	$\frac{1}{9}$ inch
Width of mouth	$2\frac{1}{2}$ inches
Distance from teeth to epiglottis	$3\frac{1}{2}$ inches
Capacity of mouth and pharynx	5 Ko (106)
Weight of tongue	10 oz.
Length of tongue	7 inches
Width of tongue	$2\frac{1}{2}$ inches
Weight of esophagus	10 oz.
Width of esophagus	$1\frac{1}{2}$ inches
Length of esophagus	1 ft. 6 inches
Length of stomach stretched out	2 ft. 6 inches
Circumference of stomach	1 ft. 5 inches
Diameter of stomach	5 inches
Capacity of stomach (dry measurement)	3 tou (107) 5 sheng (108)

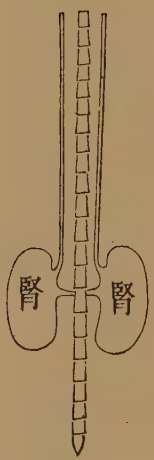
[2] Unfortunately, we cannot ascertain their equivalents in present-day standards.

13



14

15



16

Fig. 13 The three burning spaces (2697 B.C.).
Fig. 14 Stomach.
Fig. 15 Gall-bladder.
Fig. 16 Kidney.

Circumference of small intestine	2½ inches
Diameter of small intestine	$\frac{8}{10}$ inch
Length of small intestine	33 ft.
Capacity of small intestine (dry measurement)	2 tou, 5 sheng
Circumference of large intestine	4 inches
Diameter of large intestine	1½ inches
Length of large intestine	20 ft.
Capacity of large intestine (dry measurement)	1 tou
Circumference of rectum	8 inches
Diameter of rectum	2½ inches
Length of rectum	2 ft. 8 inches
Capacity of rectum	2½ Ko

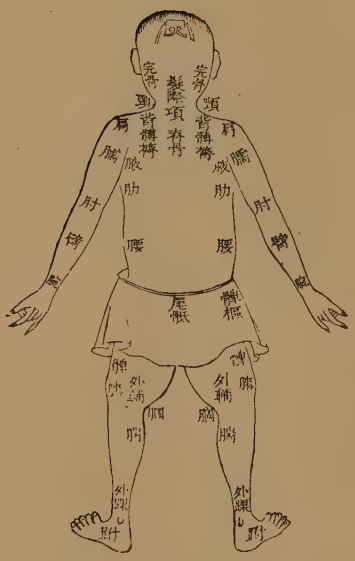
The small intestine is attached to the spine posteriorily, and anteriorily to the navel. It exhibits sixteen loops. The large intestine lies on the left side of the navel and has also sixteen curves.

In the book called *Nanching*, or *Hard Classics*, written by Chin Yüeh Jen in the Chow dynasty, 1122 B.C., we find the morphology and weights of the different organs and viscera described. It differs from *Neiching* in the following particulars:

Liver, three left lobes and three right lobes	4 catties, 4 oz.
Heart, seven holes and three pillars contains 3 Ko of vigorous principle	12 oz.
Spleen, flat, 3 inches thick, 5 inches long, contains ½ catty of loosening extract	3 catties, 3 oz.
Length, eight lobes	3 catties, 3 oz.
Kidneys, two in number	1 catty, 1 oz.
Gall-bladder rests on the short lobe of the liver, contains 3 Ko of essential fluid	3 oz. 3 drams
Stomach	2 catties, 2 oz.
Small intestine	2 catties, 14 oz.
Large intestine	2 catties, 12 oz.
Bladder when stretched 9 inches long, contains 9 sheng, 9 Ko of urine	9 oz. 2 drams
Trachea, 2 inches wide, 1 ft. 2 inches long, in 9 sections	12 oz.
Rectum	12 oz.

In the Tang dynasty, 620 A.D., Sun Ssu Miao wrote two books in which he followed the descriptions given in the *Nanching* over 1700 years earlier with but slight modifications. However, the weights of the intestine and stomach and the length of the intestine differ somewhat from those given by Huangti in his

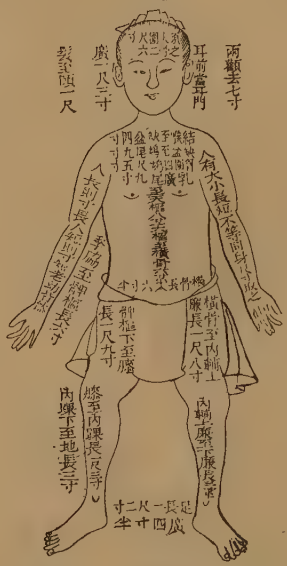
17



18

骨度側面全圖

19



20

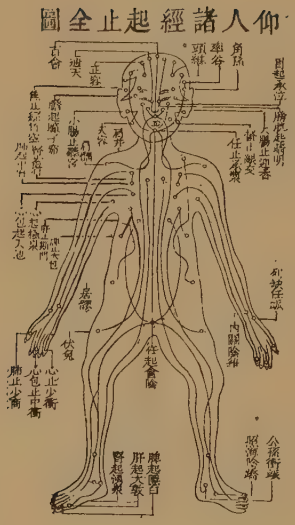
Fig. 17 Surface anatomy, letters indicate the bones, front view (2697 B.C.).
Fig. 18 Ditto, back view.
Fig. 19 Ditto, side view.
Fig. 20 Body measurements, front view.

Neiching, 2697 B.C. Sun Ssu Miao described two bladders, one receiving the 'essential fluid,' and the other, fluid and urine. In his books each organ or viscus had attached to it the name of a protecting god. This indication of theocratism is not, however, found in later writings on Chinese medicine. Controversy has been active regarding the three burning spaces (fig. 13) for upward of four thousand years. In the *Nanching* we read that the three burning spaces have no form, that they control the air (vigor), and that the two kidneys should be classed as two separate and distinct organs, the left being the real one and the right the 'gate of life.' The 'gate of life' is said, in the male, to contain the semen and, in the female, the uterus. This conception is entirely different from that advanced in the *Neiching*. Huangti, 2697 B.C., Wang Shu Ho (109), Huei Tsung (110) and Sun I Kuei (111), all contend that the burning spaces exist only in name, but not in form (108 and 109).

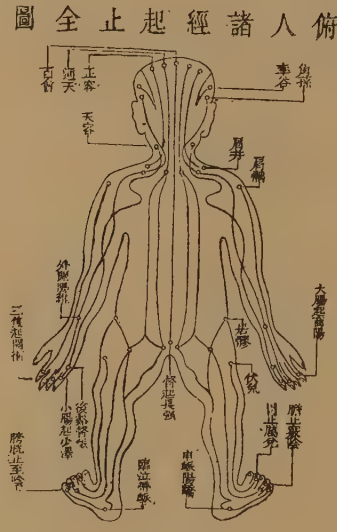
The second theory assumes that the burning spaces exhibit definite structure. This view is actively supported by Hsü Tun (112) and Chen Wu Tse (113) in the Sung dynasty. The former observed a piece of fatty membrane, about the size of a man's hand, near the bladder and two whitish cords emerging from it and running to the brain. He concluded that the membrane constituted the burning spaces (114). In the Ming dynasty, 1368 A.D., Yü Po (115) writes that the fatty membrane in the thorax also belongs to the three burning spaces. Chang Chi Pin holds the view that the thorax and abdomen are constructed like a large sac (116), that the inner lining is very red and that these linings are in truth the burning spaces. He debates the subject at length.

According to a third theory, the three burning spaces are capable of subdivision into upper and lower parts governing corresponding regions of the body. This idea is due to Li Kao (117) writing in the Yuan dynasty, 1280 A.D. (118–119). Later in the Ming dynasty, Ma Shih (120) described the three burning spaces as being a compound of two sets, one without form and the other with form. He says in his book (121) that the upper, middle, and lower burning spaces, described in *Nanching*, are

21



22



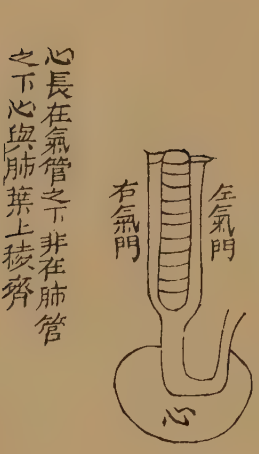
23

Fig. 21 Body measurements, back view (2697 B.C.).
Fig. 22 Traveling vessels with their needling points, front view.
Fig. 23 Ditto, back view.

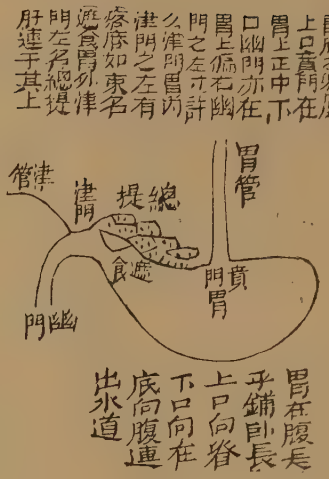
without form and filled with air, while the three burning spaces of the hand, foot, and young male have form.

A few words may here be said about the term 'Mingmen,' or 'gate of life' (122), which is first used in the *Nanching*, 1122 B.C. The still older book I-Ching (123), originally written by Fuhsi (124) in 2852 B.C. and revised by Wen Wang (125) in 1122 B.C., says that "Keep in the learning of water, to which the kidney belongs, and you will be strong" (126). Hoa-show (127) claims, in the Yuan period, 1280 A.D., that the air of the 'gate of life' connects with the kidney. We find that later, in the Ming period (1368 A.D.), Chao Hsia Ke (128) advanced evidence that the 'gate of life' is located at the needling point in the lumbar region, and further that female water belongs to the right kidney and male water to the left, between which there is a space of $1\frac{1}{2}$ inches. This area he believed to be the 'palace of life' from which the true fire, which is without form, is sent (129). But Sun I Kuei (130) expressed a different view, according to which the 'gate of life' is the original or mobile air of the body which plays between the two kidneys (131). Chang Chi Pin is a little more specific in his theory that the 'gate of life' corresponds to the orifice of the uterus where the kidney stores its essence. In Taoism this location is called 'Tautien' (132). According to still another view, the 'gate of life' is at the back opposite to and at the same level as the navel (133). All these conceptions indicate that the men of the time were trying to reverse the error of the *Nanching* which described the 'gate of life' as resident in the right kidney.

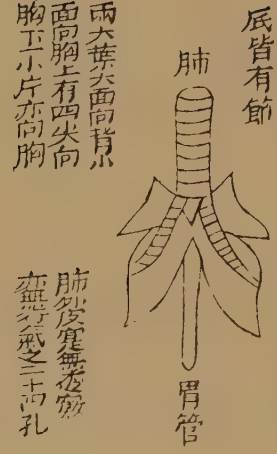
Between the Sung and Yuan dynasties, 960–1280 A.D., there is but little improvement in the science of splanchnology. The publication of Chao Hsien Ke's (134) *Morphological Atlas* (135) and of Sun I Keui's book on *Human Internal Organs* (136), mark, however, a certain advance. Subsequently, in the Ching period, 1644 A.D., Feng Chiao Chang (137) reversed the teachings of Chao and improved his descriptions. In Feng's book we read that the lung is attached to the third vertebra and hangs down in four lobes which are gray in color, that it possesses twenty-four lobes in order to provide for the circulation of air



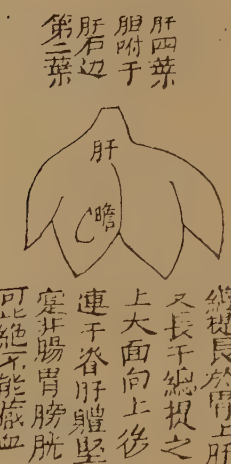
24



25



26

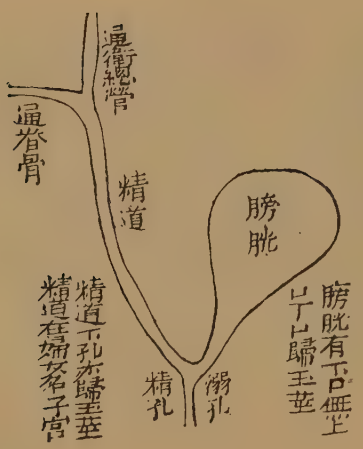


27

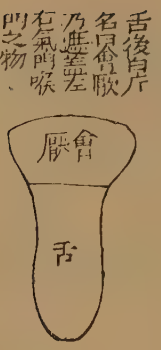
Fig. 24 Heart and trachea, Wang-ching-jen's personal drawings (1796 A.D.).
Fig. 25 Stomach, pancreas, and esophagus.
Fig. 26 Trachea, lung, and esophagus.
Fig. 27 Liver with gall-bladder.

from the various organs, that it is empty as a beehive with no opening below, and that it fills on inhalation and empties on exhalation. The spleen is attached at its upper left end a little below the eleventh vertebra, it is sickle-shaped, and is enveloped by the same membrane as the stomach, and moves when a voice is heard, grinding on the stomach, and aiding in transportation and digestion. The pericardium is situated above the transverse membrane (diaphragm), with which it is connected, and below the vertical membrane, it has yellow fat and encloses the heart, to which it is connected by a thin fibrous membrane-like silk. The heart is attached to the fifth vertebra lying below the tube of the lung and above the diaphragm; it is conical in shape like a lotus bud, with openings at the side, but none below; it shows some individual variation, being connected with the tongue above and by means of four cords with the lung, spleen, liver, and kidneys. The diaphragm is attached to the ribs and to the spine and prevents the impure air from coming up from below. The bladder lies at the level of the nineteenth vertebra below the kidneys and in front of the large intestine; it possesses an opening below, but none above. Feng remarks that according to some books the bladder has an upper opening only and that others say that it has no opening at all, opinions which he considers to be quite erroneous. He believes that the urine is produced through a process of air change in the body. The kidneys are two in number, each the shape of a bean, one on either side of the spine, and separated by about $1\frac{1}{2}$ inches. They are enveloped in yellow fat and each is supplied with two cords which run up and down. The upper pair are attached to the heart and the lower pair run down along the spine, pass over the coccyx, where they enter a space about half the size of a man's hand; they leave this space through two holes and pass upward along with the spinal cord to the brain (138).

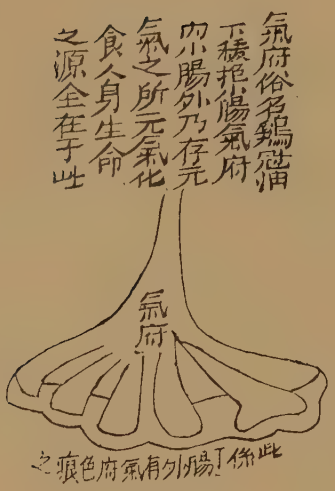
In the Ching dynasty (1644 A.D.), during the days of Chia Ching and Tao Kung (1796–1821 A.D.), there lived a Chihli medical man, Wang Ching Jen, who spent forty years in the study of splanchnology and wrote a book entitled *Corrections of Faults in Medicine* in order to overthrow the teaching in the



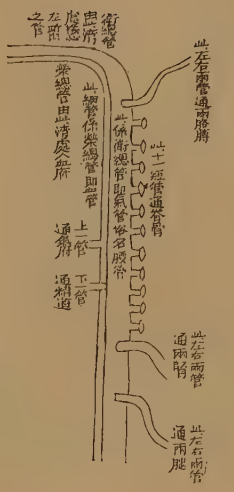
28



29



30



31

Fig. 28 Bladder, urethra, and vas deferens. Wang-ching-jen's drawing (1796 A.D.).
Fig. 29 Tongue and epiglottis.
Fig. 30 Omentum.
Fig. 31 Vena cava and aorta.

Nanching. Wang made use of his exceptionally good opportunities for study. His book is based upon twenty-four corrected pictures of the organs and viscera, of which figures 25 to 31 will serve as examples, and contains the following five essays:

1. *The Epiglottis, Right and Left Air Gates: the Main Protective Tube, the Main Nourishing Tube; and Air and Blood Centers.*
2. *The Route of Digestion and the Excretory System.*
3. *The Brain.*
4. *The Air, the Blood, and the Pulse.*
5. *The Absence of Blood in the Heart.*

We may mention in conclusion Peng Tsung Hai (139), from the province of Ssuchuan, who wrote during the Kwang Hsu period, 1875 A.D. He was a follower of Wang and was fortunate in being able to secure an atlas of western medicine for comparison in testing out the theories in *Neiching*. Nevertheless, he holds to the old views and describes the 'gate of life' as corresponding to the cords of the kidney and the fat lying below the cords as the lower burning spaces. The fat in the region of the omentum corresponds in his mind to the middle burning space and that above the diaphragm to the upper burning space. He describes that part of the bladder which connects with the fat as the place where the water enters. And finally he seems convinced that the brain carries out the action of the heart, which is in direct opposition to the theories of Chin Cheng Hsi (140) and Li Chih Chen (141), the former being of the opinion that the brain is the seat of memory and the latter that it is the 'palace of God' (142, 143).

HISTORY OF CHINESE ANGIOLOGY

Angiology and osteology are the only sciences in which the arts of acupuncture, cautery, and osteopathy are used therapeutically. During the time of Huangti, 2697 B.C., acupuncture was used extensively as a method for the treatment of disease (*Neiching*, vol. 14), but just how the practice originated we cannot tell. We have read about the relations of the five organs to the different parts of the body, how the skin relates to the

lung, muscle to spleen, vessel to heart, ligament to liver, and bone to kidney; now, when the needle punctures the different tissues at various depths, there will be a reaction on the organ to which the tissue is related.

The vascular system makes connections between the organs and viscera which are placed inside the body and all the other parts.

The traveling vessels circulate the blood and the air to nourish the male and female principles, to moisten the bones and ligaments, and to lubricate the joints. Twelve pairs are recognized with their respective branches (figs. 22 to 23).

1. The hand great female lung vessels from the middle burning space to the tip of the thumb.

2. The hand male proper large intestine vessels from the tip of the thumb and of the small finger to the large intestine.

3. The foot male proper stomach vessels from the middle of the nose to the middle toe of the foot.

4. The foot great female spleen vessels from the great toe to the lower part of the tongue.

5. The hand young female heart vessels from the heart to the inside of the little finger.

6. The hand great male small intestine vessels from the little finger to the small intestine.

7. The foot great male bladder vessels from the inner corner of the eye to the little toe of the foot.

8. The foot young female kidney vessels from the little toe to the root of the tongue.

9. The hand female proper pericardium vessels from the middle of the stomach to the tip of the middle finger.

10. The hand young male three burning space vessels from the tip of the little finger to the three burning spaces.

11. The foot young male bladder-vessels from the outer angles of the eyes to the little toes.

12. The foot female proper vessels from the hairy spots on the big toes to the vertex of the head connecting with the central vessels (144).

There are also eight singular traveling vessels as follows:

1. The Chung Moa (145), or 'ventral rising vessel.'
2. The Jen Moa (146), or 'responsible vessel.'
3. The Tu Moa (147), or 'governor vessel.'
4. The Tai Moa (148), or 'girdle vessel.'
5. The Yin Chiao Moa (149), or 'outer heel vessel.'
6. The Yang Chiao Moa (150), or 'inner heel vessel.'
7. The Yang Wei Moa (151), or 'external longus vessel.'
8. The Yin Wei Moa (152), or 'internal longus vessel.'

Though the twelve pairs of traveling vessels are separated in their course, six regular anatomoses occurring in the extremities are recognized:

1. The foot great male with foot young female.
2. The foot young male with foot female proper.
3. The foot male proper with foot great female.
4. The hand great male with hand young female.
5. The hand young male with the heart.
6. The hand male proper with the hand great female.

The traveling vessels are usually deeply placed and concealed in the muscles, but one pair, as it passes the external malleolus, lies superficially and can easily be seen to pulsate. Each vessel has definite places in its course where the pulse air may be let out. Some of them have more than others, but the total number is 365. These places are called the 365 needling or puncture points (153).

In addition to the twelve pairs of traveling vessels, we have twelve pairs of traveling ligaments, following the same course, to deal with. The ligaments are more closely associated with the limbs, muscles, and joints (154). The pulsating vessels are not able to pass over the larger joints, where they turn to the skin and make their connections so that we are unable to trace their pulsations. They are fifteen in number (155).

The stomach has a large vessel for itself. The branches of the vessels are called *Sun* (156), or descendents. They serve for the overflow of the strange and malicious excess and for the conveyance of nourishment and protection. The large junctions of the vessels with the muscles are called *Hui* (157) and the smaller ones *Hsi* (158). In the muscular fibers and the muscular

junctions there are routes for nourishment and protection, where the air meets. To correspond with the needling points 365 small branches of vessels and 365 muscular junctions are recognized (159). Each of the five organs has five traveling vessels, making in all twenty-five small traveling vessel spots; and each of the six viscera six traveling vessels, making thirty-six more small traveling vessel spots.

Counting the twelve traveling vessels and the fifteen pulsating vessels, we have altogether twenty-seven air routes which go up and down. The site where each starts is called the spring, the current carries nutrition which flows toward the termination or needling spot.

There are likewise 365 articulations which receive air in the following manner. The five organs have six viscera, and the six viscera twelve springs whence the air circulates through the four passes (at shoulders and hips) and is distributed directly to the joints.

With reference to the pulse, we read that in the normal condition there is one beat for each expiration and inspiration. When each expiration has three beats and each inspiration four it is said to be the pulse of death (160).

When we remember that this complicated system which we have outlined has been handed down to us from Huangti, over four thousand years ago, we cannot help admiring the thoroughness and ingenuity which has been displayed. The obvious inconsistencies and contradictions are surely to be expected.

In the Tang period, 620 A.D., Sun Ssu Miao collected the atlases used by prominent doctors and wrote an interesting book on *Acupuncture and Cautery*. He made illustrations in five colors showing 650 needling points in three planes (front, back, and side views). He listed 345 special names for the different points (161). Unfortunately, the original pictures in his atlas have been lost. A few years later Wang Tao (162) revised the atlas and made twelve pictures corresponding to those in the books of Huangti, Sun, and others (163). We still have the descriptions, but these illustrations have also been lost.

In the Sung dynasty (960 A.D.) the Emperor Jen Tsung (164) ordered Wang Wei I (165) to construct a bronze figure in which all the old theories of anatomy were to be corrected and incorporated. Holes were drilled to represent the needling points, in accordance with the descriptions of Sun Ssu Miao (166). The interior of the figure is fitted with models of organs and viscera surrounded with water.

During this dynasty two important works were written by Hsi Fang Tzu (167) entitled *Needling Cautery Classics on the Bronze Figure* (168) and *Ming Tang Needling Cautery Classics* (169). About half the needling points mentioned differ from Sun's descriptions. Several other papers of less importance dealing with acupuncture and cauterization appeared at this time, which it will be hardly worth while to consider here.

Yang Chi Chow (170) collected all the old records and compiled a system of acupuncture and cauterization (171) in the Ming dynasty, 1468 A.D., which deserves mention before concluding this section.

ANTHROPOLOGY

The measurements which I have already given (pp. 107, 109) will indicate clearly the intense interest which the old Chinese anatomists took in determining physical standards. Huangti, many years ago, made a conscientious attempt to ascertain and record the average measurements of the men of his day. He said to his servant Po-Kao (172): "I desire to know the average length of the bones if a person has a height of seven feet and a half." Unhappily, the standard has been lost, so that we cannot ascertain the absolute value of the feet and inches used as units by Huangti, but the figures still possess a certain relative value. In answering, Po-Kao gave the following measurements (figs. 20 and 21):

Head circumference	2 ft. 6	inches
Thorax	1 ft. 5	inches
Hairy area of scalp	1 ft. 2	inches
Vertex of head to angle of mandible	1 ft. 0	inch
Thyroid eminence to interclavicular notch	2	inches

Sternum length	9 inches
Sternum to umbilicus	8 inches
Umbilicus to transverse bone (pubes)	6 inches
Width of transverse bone	6½ inches
Upper border of the pubes to upper border of internal condyle	1 ft. 8 inches
Upper border of internal condyle to its lower border	3½ inches
Lower border of internal condyle to internal malleolus	1 ft. 3 inches
Internal malleolus to sole	3 inches
Back of knee-joint to foot, dorsal surface	1 ft. 6 inches
From dorsal surface of foot to ground	3 inches
External angle of frontal bone to the clavicle	1 ft.
Clavicle to axillary space	4 inches
Axillary space to twelfth rib	1 ft. 2 inches
Twelfth rib to hip-joint	6 inches
Hip to the middle of the knee	1 ft. 9 inches
Knee to external malleolus	1 ft. 6 inches
External malleolus to calcaneus	3 inches
Calcaneus to ground	1 inch
Between two mastoids	9 inches
Between two ears	1 ft. 3 inches
Between two malar prominences	7 inches
Between two hips	6½ inches
Foot length	1 ft. 2 inches
Foot width	4½ inches
Shoulder to elbow	1 ft. 7 inches
Elbow to wrist	1 ft. 2½ inches
Wrist to first joint of middle finger	4 inches
First joint of middle finger to tip of finger	½ inch
Border of hair of scalp to seventh vertebra	2½ inches
Seventh vertebra to sacrum	3 ft.

In the Sung period, 960 A.D., the emperor ordered his physicians to edit a system of medicine (173), in which we can find some further measurements, but unhappily the method of making them has not been described. We read that there are 365 bones in the male and five less in the female, that 190 of them are concealed so that they cannot be seen and that 256 possess bone-marrow.

The works of Huangti remain perhaps the most complete on record. He is certainly to be regarded as the father of Chinese medicine.

CONCLUSIONS

It is quite evident from the foregoing account that in the beginning the science of anatomy in China was based upon actual dissection of the human body.

The most notable evidence in favor of this conclusion is briefly as follows: Chipo, the servant of Huangti, 2697 B.C., writes that "after death the body may be dissected and actual observations made." A little later we read that Yin Chow (174), 1122 B.C., killed Pi Kan (175) and dissected his heart to discover whether it had seven openings. In the Han period, 206 B.C., Wang Mang (176) slew Chen Hsun (177) and dissected his arm; the same gentleman also captured a revolutionary and ordered his physicians to dissect him.

We find, also, that executions were frequently adapted to anatomical purposes. For instance, Liang Shao Pao (178), 960 A.D., sent his medical officer with an artist to make pictures during an execution of thieves, probably by the rather slow slicing process; and the local officer, Li I Hang (179), employed doctors and artists and labored himself to make dissections and to correct drawings during an execution at Ssu Chow. Yang Chik (180) compared these drawings with the old books, found them to be correct, and accordingly constructed his famous *Atlas of Truth.*

We have already mentioned the terrible epidemic which raged among the children in the town of Chang Li Hsieh in the days of Chia Ching (1796 A.D.), and of how a certain magistrate, named Wang Chin Jen, happened to visit the public cemetery where he found that hungry dogs were uncovering the bodies, hastily buried in shallow graves, and devouring them. Wang's curiosity was so aroused that he went daily to the cemetery and observed over thirty complete bodies dismembered. He was thus enabled to test out the old theories and to make important new observations which formed the basis for his book called *A Correction of Faults in Medicine.*

Unfortunately, this direct method was soon replaced by a rule of authority somewhat similar to that which reigned in Europe

before the Renaissance. For instance, *The Essentials of a Gold Medicine Case* and a book on *Fevers*, written by Chang Chi in the Han dynasty (206 B.C.), remain very popular to this day. With the reintroduction of direct observation and a true appreciation of the value of experimental methods, we may look for great advances.

Bibliography

1. 內經 Huangti (2697 B.C.)
2. 素問 Huangti (2697 B.C.)
3. 靈樞經 Huangti (2697 B.C.)
4. 難經 Chin Yüeh Jen (1122 B.C.)
5. 甲乙經 Huang Fu Mi (265 A.D.)
6. 素問全遺 Wang Su Ho (265 A.D.)
7. 千金要方 Sun Ssu Miao (620 A.D.)
8. 外台秘要 Wang Tao (620 A.D.)
9. 千金翼方 Sun Ssu Miao (620 A.D.)
10. 銅人腧穴鉄灸圖經 Sun Ssu Miao (620 A.D.)
11. 蘇黃門龍川志 Suhuangmen (960 A.D.)
12. 三因方 Chen Wei Tsai (960 A.D.)
13. 聖濟總錄 Sung period (960 A.D.)
14. 此事難知 Wang Hao Wen (1280 A.D.)
15. 難經說 Hua Show (1280 A.D.)
16. 醫学正傳 Yu Fu (1368 A.D.)
17. 類經附翼 Chang Chi Pin (1368 A.D.)
18. 難經正意 Ma Shih (1368 A.D.)
19. 醫貫 Chao Hsia Ke (1368 A.D.)
20. 醫旨緒餘 Sun I Kuei (1368 A.D.)
21. 鍼灸大成 Yang Chi Chow (1368 A.D.)
22. 錦囊秘錄 Feng Chiao Chang (1644 A.D.)
23. 醫宗金鑑 Chien Lung (1736 A.D.)
24. 醫林改錯 Wang Ching Jen (1796 A.D.)
25. 中西醫学會通 Peng Tsung Hai (1875 A.D.)

No. 1 太嚳
2 飛龍
3 黃帝
4 倉頡
5 堯
6 禹
7 秦
8 李斯
9 大篆
10 小篆
11 秦篆
12 程邈
13 隸書
14 王倫
15 漢
16 賈魴
17 三倉
18 蔡邕
19 石經
20 漢隸
21 晉
22 鍾繇
23 王羲之
24 岐伯
25 內經
26 素問
27 靈樞經
28 唐
29 王冰
30 周
31 秦越人
32 難經
33 張機
34 金匱要畧
35 傷寒論
36 肘後備急方
37 葛洪
38 晉
39 褚澄
40 遺書
41 巢元方
42 隋
43 孫思邈
44 王燾
45 唐
46 王冰
47 沈括
48 陳直
49 董汲
50 劉溫
51 韓祇
52 龐安時
53 攺和中
54 唐慎
55 王貺
56 許叔
57 陳師文
58 夏德
59 張杲
60 陳自明
61 陳言
62 李迅
63 嚴用和
64 楊士瀛
65 宋
66 劉完素
67 張元素
68 張從正
69 李杲
70 金
71 王好古
72 沙圖穆蘇
73 危亦林
74 朱震亨
75 王國瑞
76 齊德之
77 載攺宗
78 王履
79 元
80 張介賓
81 明
82 類經
83 清
84 乾隆
85 醫宗金鑑
86 沈彤
87 釋醫
88 嘉慶
89 昌黎縣
90 王清任

91 医林改错
92 太阴
93 厥阴
94 少阴
95 太阳
96 阳明
97 少阳
98 表裏
99 藏
100 腑
101 曲
102 徵
103 宫
104 商
105 羽
106 合
107 斗
108 升
109 王叔和
110 徽宗
111 孫一奎
112 徐遁
113 陳無擇
114 蘇黃龍川法三因方
115 虞博
116 類經附翼
117 李果
118 此事難知
119 王好古
120 馬蒔
121 難經正義
122 命门
123 易經
124 伏羲
125 文王
126 難經說
127 浩壽
128 趙獻可
129 医貫
130 孫一奎
131 医旨緒餘
132 毋田
133 類經附翼
134 趙獻可
135 形景芳流
136 身内景說
137 馮兆張
138 錦囊秘錄
139 彭宗海
140 金正希
141 李時珍
142 本草備要
143 中西医学滙通精義
144 靈樞經脈篇
145 衝脈
146 任脈
147 督脈
148 帶脈
149 陰蹻脈
150 陽蹻脈
151 陽維脈
152 陰維脈
153 素问气府論
154 靈樞經筋篇
155 靈樞經流篇
156 孫
157 會
158 絡
159 气穴論
160 素问脈篇
161 千金要方
162 王燾
163 外台秘要
164 仁宗
165 王惟一
166 孫思邈
167 西方子
168 銅人腧穴鍼灸圖經
169 銅人鍼灸經
170 楊繼洲
171 鍼灸大成
172 伯高
173 聖濟總錄
174 殷紂
175 比干
176 王莽
177 甄尋
178 梁少保
179 李夷行
180 楊介

Resumen por el autor, Oliver H. Gaebler
Universidad de Missouri.

El epitelio de la vejiga durante la contracción y la distensión.

El presente trabajo trata del problema de sí durante el proceso de distensión fisiológica, el numero de capas celulares del epitelio de la vejiga permanece constante o, por el contrario, si disminuye, es decir, si las células epiteliales simplemente se aplanan o si se disponen en un número menor de capas. En los estudios llevados a cabo, el autor ha empleado material procedente de ratas, conejillos de indias, y conejos, pero el trabajo más importante fué hecho en conejos.

Para resolver el problema se han empleado dos métodos. El primero consiste en contar el número de capas del epitelio en vejigas en diversos grados de distensión, comparando dichas capas con el número encontrado en vejigas contraidas. El segundo método empleado depende del hecho de que sí no hay una disposición de las células epiteliales en un número menor de capas, la relación que representa el aplanamiento del epitelio en cualquier dirección debe ser idéntica a la que representa el aplanamiento de sus células en la misma dirección. Estas relaciones se determinaron mediante medidas del epitelio y de diez mil células, en dos series de vejigas de conejos.

Las conclusiones obtenidas por ambos métodos son: 1) La distensión fisiológica moderada no disminuye el número de capas de células epiteliales; 2) La distensión fisiológica máxima no disminuye las capas citadas en mas del 12.5 por ciento; 3) Las células de las capas más profundas no están unidas tan íntimamente como las de las capas superficiales, y 4) Las células no sufren una contracción ulterior después que ha comenzado el plegamiento del epitelio.

Translation by José F. Nonidez
Cornell Medical College, New York

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, NOVEMBER 15

BLADDER EPITHELIUM IN CONTRACTION AND DISTENTION

OLIVER H. GAEBLER

Anatomical Laboratory, University of Missouri

NINE FIGURES

The main problem with which this paper deals may be stated in the following questions: Does the epithelium of a bladder in distention have the same number of layers of cells as the epithelium of a similar bladder in contraction? Does the process of distention involve only a stretching of the cells, or also an arrangement into fewer layers?

This problem has been discussed for many years. London ('81) studied the relation between thickness of the epithelium and degree of distention of the bladder, because he believed that data obtained would be of interest in connection with the problen of resorption of substances from the urine. Since the epithelium of the bladder forms a barrier between the contained urine and the capillaries in the underlying connective tissue, the thinning out of this epithelium on distention was thought to be significant in connection with the stated problem. In regard to the number of layers of cells, London ('81) said that there was an apparent diminution during distention, but an actual diminution only in the number of layers of nuclei, and not in the number of layers of cells.

Dogiel ('90) studied the histology of bladder epithelia of mice, rats, and various other mammals, and claimed that the cells of the first two layers, counting from the surface layer, were so interlocked by protoplasmic processes that there was no possibility of a change in their relative position occurring during distention.

Eggeling ('01–'02) and many others published articles on the histology of bladder epithelium, but did not touch upon the

problem under discussion in this paper. Harvey ('09) described the variations in all the layers of the walls of both bladder and ureter during contraction and distention. In regard to the bladder epithelium, he says that there is a decrease of 50 per cent in the number of layers of nuclei during distention, and also a decrease in the actual number of layers of cells.

Concerning the human bladder, one histologist (Lewis and Stöhr, '13, p. 324) says the following: "The epithelium has been described as two-layered in the distended bladder, the outer cells having terminal bars; in the contracted condition it becomes several layered, and the bars form a net extending into the epithelium. Thus it is not believed that during distention the layers merely flatten; they are thought to 'slip by each other.' The columnar cells may, however, become extremely flat."

The various observers, using material from many sources, and employing numerous methods, have, therefore, reached several conclusions that bear upon the particular problem here discussed. Some of them have pointed out the decrease in the number of layers of nuclei in bladder epithelium during distention and have determined this decrease quantitatively. Some have stated that while there is a decrease in the number of layers of nuclei, there is no decrease in the actual number of layers of cells. Others have claimed that there is an actual decrease in the number of layers of cells during distention, but have made no statements regarding the extent of this decrease. The following studies were therefore made for the purpose of finding out whether there is any diminution in the number of layers of cells during distention, and, if so, how great the diminution is.

MATERIALS AND METHODS

In the experiments that follow the animals used were white rats, guinea-pigs, and rabbits. The preliminary work, done on some material from each of these three sources, suggested that the results would be very much alike, if not identical, for the three animals, and since rabbit material seemed the most favorable for the particular methods of procedure and staining required, the final work was done on rabbit material.

In obtaining bladders in various degrees of contraction and distention, it is evident that the more nearly normal, or physiological, the process of contraction or distention, the less open to objection is the result. Bladders in various degrees of normal distention or contraction can be obtained by simply taking animals from their cages, killing them at once, and relying on chance. If chance killing does not yield a sufficient number of contracted bladders, female rabbit bladders can be made to contract by pressure on the lower abdomen or by irritation of the urethra. With male rabbits the pressure method is less reliable. If completely contracted bladders are wanting, they can be produced by cutting the urethra or opening the bladder in any way, in an animal that has just been killed. If greatly distended bladders are desired, the animals are given an abundance of water, taken from their cages and played with or excited in any way for thirty minutes to an hour, and suddenly killed.

To fix a bladder found in partial or complete distention, the urethra and ureters are clamped with one hemostat immediately after the abdomen has been opened, and are then cut on the side of the hemostat away from the bladder, so that the urine is retained. The bladder is then immersed in saturated mercuric chloride solution for two or three minutes. This destroys the contractility of the muscle cells. In guinea-pigs a longer exposure was needed to accomplish this result. The bladder is next transferred to physiological salt solution, cut open, washed out in several changes of salt solution if necessary, and returned to mercuric chloride solution for twelve hours.

In completely contracted bladders, the part used for sections is a cylindrical piece secured from the middle of the bladder by two parallel cuts, made at right angles to the long axis of the bladder and 2 or 3 mm. apart. In partly or completely distended bladders, a corresponding equatorial zone, about 5 mm. wide, is cut out after fixation. This zone is cut open anywhere, measured just before imbedding, and cut into a convenient number of pieces, all of which are imbedded.

The solution of the problem depends fundamentally upon an unmistakable staining of the cell boundaries. Such staining will

prevent overlooking of cells whose nuclei are not included in the section, and will enable one not only to count the number of layers of cells, but also to measure accurately the size of the cells. A number of staining methods were tried, but it was found that the following iron hematoxylin methods, when used with rabbit material, were the most successful:

First method. 1. Fix in a saturated solution of mercuric chloride in physiological salt solution.

2. Stain with freshly prepared Hansen's hematoxylin to which no sulphuric acid has been added (Lee, '13, p. 159) until the sections are very black. This requires fifteen to thirty minutes. Decolorize in 2.5 per cent iron alum solution, to the point where cell boundaries show plainly, paying no attention to the layers of the bladder wall other than the epithelium. Clear and mount without counterstaining.

Second method. Mallory's chloride of iron hematoxylin method, as described in Mallory and Wright's 'Pathological Technique,' page 310. In differentiating, again watch the epithelium only.

Although the solution of the problem depends fundamentally upon the success of the foregoing procedures, one very important complication remains. Due to the complexity of the folding of the epithelium of completely contracted bladders, sections taken at right angles to the long axis of the bladder will be perpendicular to the epithelium at only a few points, and tangential everywhere else (fig. 9). This difficulty is avoided by securing bladders that have contracted just to the point where folds begin to form, and fixing them in this condition by the method previously described. Sections can then be obtained that run perpendicular to the surface of the epithelium, just as carefully prepared sections of distended bladders do. This simplifies the entire problem a great deal.

Although important results were obtained from rat and guinea-pig material, the results obtained from six rabbits will cover all the facts ascertained, so it will be best to give the details of this part of the work, and pass by the remainder to avoid repetition.

Six rabbits, all of the same litter, all female, and a little less than half grown, were dealt with as follows:

Rabbit no. 1. Weight, 26 oz. The rabbit was well supplied with water, Tuesday, January 6, 1920; taken from cage at 8:30 P.M., played with for half an hour, and killed by a blow at 9 P.M. The abdomen was opened. The bladder was found widely distended, was clamped off, removed, and suspended in bichloride solution for two minutes. Its equator measured 10.4 cm. It was suspended in normal saline while the base was cut off. No contraction followed. The bladder was washed out and replaced in bichloride at 9:10 P.M.

Rabbit no. 2. Weight, 26 oz. This rabbit was taken from the cage, January 6, 1920, 9:15 P.M. Pressure was put upon the lower abdomen at once, and resulted in passage of a small amount of urine. The animal was left for a minute, and was then killed by a blow. The abdomen was opened, and the bladder found completely contracted. The bladder was removed, washed out with physiological salt solution, and put in bichloride at 9:20 P.M.

Rabbit no. 3. Weight 26 oz. This rabbit was killed Saturday, January 24, 1920. The procedure and results were very similar to those for rabbit no. 1, excepting that the distention was smaller. The equator of the bladder measured 8.2 cm.

Rabbit no. 4. Weight, 26 oz. The rabbit was killed Saturday, January 24, 1920. Procedure and results were very similar to those for rabbit no. 2. The bladder was not found completely contracted, but contracted completely when the base was cut.

Rabbit no. 5. Weight, 24 oz. The bladder of this rabbit was partly emptied by pressure on the abdomen. The animal was then killed by a blow, and the bladder was found partly contracted. It was clamped off and removed, suspended in bichloride three minutes, suspended in normal saline while the base was cut off, and was then washed out. The folds in the epithelium had just begun to form in one region of the bladder. The bladder was replaced in the fixative within ten minutes after the death of the animal, January 26, 1920.

Rabbit no. 6. Weight, 24 oz. The rabbit was killed on February 1, 1920. The procedure and results were practically identical with those for rabbit no. 5.

The sections were all cut 10μ thick. While this is thicker than is usually recommended for the methods of staining used, staining of cell boundaries in the epithelium is in no way interfered with, and sections of this thickness were valuable because the relations in any one focal plane could be more firmly established by focusing at various depths.

RESULTS OBTAINED BY COUNTING THE NUMBER OF LAYERS OF EPITHELIAL CELLS

After all these specimens had been sectioned, two methods of approaching the problem were used. The first, and most obvious, was simply to count the number of layers of cells in the epi-

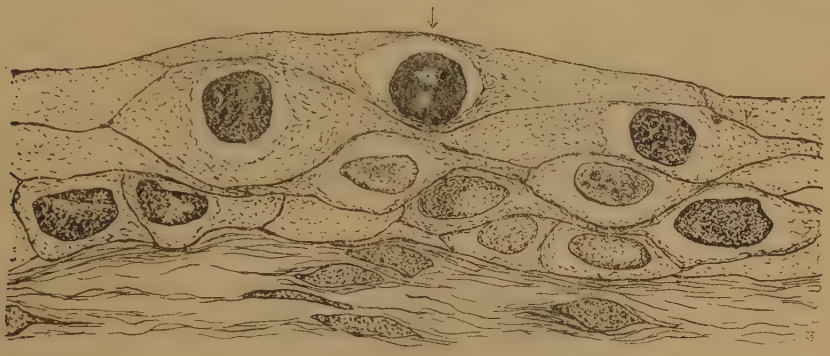
thelia of the various bladders. In the completely contracted bladders, with complexly folded epithelia, the number of layers varies a great deal, due to the great number of places where the section is tangent. But at frequent intervals the number of layers is three to four, and if the number of layers is approximately the same over the entire bladder, these places must be the points where the section is cut perpendicular to the surface of the epithelium, and hence the points giving the correct number of layers. The number of cells in sections from bladders that had contracted just to the point where folds began to form was also counted, and in these there were regularly three to four layers. The number of layers in the distended bladders was also three to four. In all cases the variation was slightly greater than this, for there were points at which only two layers of cells could be distinguished, and others at which there were no less than six layers, but three to four layers constituted the most frequent thickness.

The sections were studied under oil immersion, at a magnification of 950. With this magnification, the cell boundaries stained by the indicated methods stand out with surprising clearness, both in the contracted and in the distended specimens. Low magnifications proved very deceptive. Several of the figures illustrate the mistakes most easily made in counting the number of layers of cells.

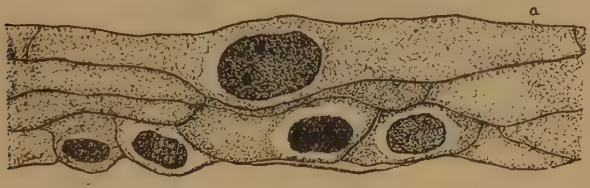
Figure 1 is a camera-lucida drawing of a portion of one of the sections from the distended bladder no. 1. At lower magnifications one would readily suppose that, counting down from the large surface cell, at the point indicated by the arrow, there were in all three layers. But at higher magnifications it is clearly seen that there is a junction of the two cells lying beneath this surface cell, and that there is a cut edge of a basal layer cell just above the nearest connective-tissue nucleus; so the number of layers in this region is five. Focusing down, the slip seen at the base in the focal plane of the drawing develops into a nucleated cell.

The method of counting nuclei was not found serviceable in determining the number of layers at any given focal plane. This is shown in figure 2, which is a camera-lucida drawing of a

portion of a section of the distended bladder no. 3. If one depended upon the nuclei, the number of layers would vary between two and one. But here again the edges of cells cut outside of their nuclei make the variation one between three and four layers. It should also be mentioned that if a line is drawn perpendicular to the surface of the epithelium at (*a*), it passes through four cells,



1



2

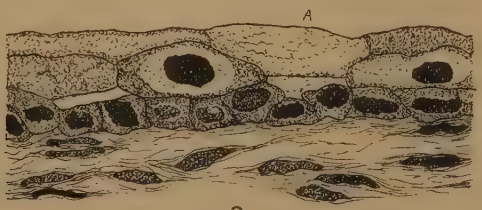
Fig. 1 Camera-lucida drawing of portion of epithelium of distended bladder no. 1 (×1128). The arrow marks the point at which the five-layered epithelium is easily mistaken for a three-layered one.

Fig. 2 Camera-lucida drawing of portion of distended epithelium of bladder no. 3 (×1128). The layers of nuclei vary between one and two. Actual layers of cells vary between three and four.

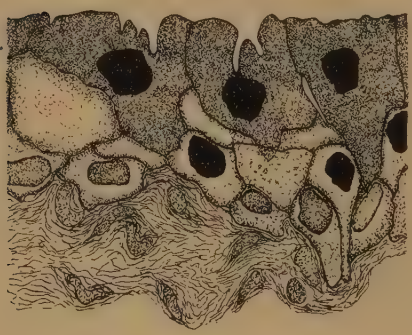
but the number of layers is really only three, since the last two cells through which the line passes are really in the same layer, the boundary between them being diagonal.

Figure 3, a camera-lucida drawing of portion of the epithelium of distended bladder no. 1, again emphasizes the fact that in this series of studies the method of counting nuclei had to be aban-

doned, because the number of layers of cells was the information desired. It also presents another example of the importance of counting cells whose cut edges might easily escape notice at low magnifications. In the region (*A*) the epithelium might easily be mistaken for a two-layered one, because the small cut edge of a second layer cell and the surface cell are about equally granular



3



4

Fig. 3 Camera-lucida drawing of portion of the distended epithelium of bladder no. 1 (× 705). The figure shows the importance of counting portions of cells not containing nuclei, such as those in the region *A*, when estimating the number of layers.

Fig. 4 Camera-lucida drawing of portion of the epithelium of contracted bladder no. 6 (× 705). This figure shows two places at which the contracted epithelium is only two layers thick.

and appear about the same shade. But a fine, distinct cell boundary separates them.

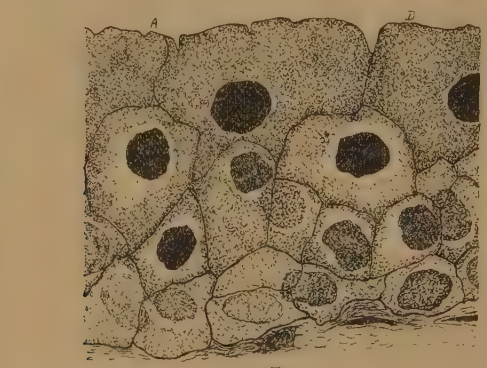
When all these precautions are observed, there will still be some points where the distended epithelium appears to be only two layers thick. But, turning to the bladders that have contracted to the point where folds just begin to form, we will find

quite as many places where the epithelium is only two layers thick. Figure 4 shows a piece of contracted epithelium, and at two places in the figure there are only two layers of cells.

There is evidence that the cells in the epithelium are bound to their neighbors pretty firmly. If one examines the various figures of contracted and distended epithelium shown in this paper, it is noteworthy that in the distended bladders the cells are still bound to their lateral neighbors along a considerable distance. In the surface layer these boundaries between cells may run perpendicular to the surface or at almost any other angle, but the length of the boundary bears about the same relation to the greatest thickness of the distended cells as the lengths of the boundaries between surface cells in the contracted epithelium bear to the greatest thickness of the cells in contraction. The surface cells with basal processes are also interesting objects in this connection. Figures 5 and 6 show portions of two bladders, one in contraction and one in distention, in which there is a comparable, though not identical relation of such cells. The three cells in the second layer, in the region between (A') and (B') of figure 6, bear about the same relation to the large surface cell in this region as the three second-layer cells in the region between (A) and (B) in figure 5 bear to the contracted surface cell with the basal processes. And the significant point in the figures is that the surface cell in figure 6, though distended to the great length of 95.7μ still has points along its lower border that suggest remnants of basal processes. Considering the degree of distention of this surface cell, one would expect its lower boundary to be more nearly straight, if there were not a firm attachment of the lower cells that exerted a tension at the two points where the most marked irregularities exist.

Sections of the extensively folded epithelium of completely contracted bladders are also interesting subjects for study. On the crest of a fold, a section through the epithelium will frequently have the appearance shown in figure 7. The appearance of the surface cells is that of partial distention, as though they had been stretched by the pushing in of the fold.

In the bottom of the pits, or rather troughs, between folds, the cells frequently have a columnar appearance. This is shown in figure 8. In the middle of the piece of epithelium shown, this columnar appearance is not only apparent, but real, while at the borders the figure is misleading, because the epithelium is rotated through ninety degrees from the position in which it is usually figured.



5

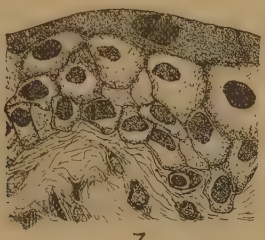
6

Fig. 5 Camera-lucida drawing of portion of the epithelium of distended bladder no. 6 (× 705). The surface and second-layer cells in the region between *A* and *B* bear a relation to one another similar to that of the corresponding cells in figure 6, in the region *A'* to *B'*.

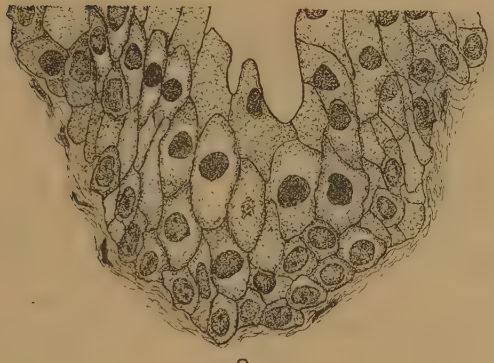
Fig. 6 Camera-lucida drawing of portion of the epithelium of distended bladder no. 3 (× 705). Note the projections on the lower surface of the large surface cell, suggesting remnants of basal processes.

The first method of approaching the problem under discussion —the method of simply counting the number of layers of cells in any given focal plane—therefore results in the conclusion that the range of variation in the number of layers in the contracted bladder is about the same as in the distended bladder. The usual number of layers in both is three to four, and in either

contracted or distended bladders there may be places where there are only two layers or where there are more than four. This method of stating results still does not give completely the information desired, for the question remains: Does the contracted bladder contain more area covered by four layers and the distended bladder more area covered by three layers? If there is



7



8

Fig. 7 Camera-lucida drawing of the epithelium at the crest of a fold in completely contracted bladder no. 2 (× 422). Note the partially distended appearance of the surface cells.

Fig. 8 Camera-lucida drawing of the epithelium in a trough between folds of completely contracted bladder no. 2 (× 422). Note the columnar appearance of the cells.

such a difference, it is not so noticeable as to be at once agreed upon after studying two comparative sections. So at this point the second method of inquiry, depending upon cell measurements is brought in.

METHOD OF CELL MEASUREMENTS, AND ITS RESULTS

It is evident that if all of the stretching of the epithelium is to be accounted for by stretching of the cells, and not by 'slipping' or rearrangement into fewer layers, the ratio that represents the stretching of the epithelium in any given direction should be identical with the ratio that represents the stretching of the cells in this direction.

The amount of stretching of the epithelia was determined as follows: After fixing, as stated before, an equatorial zone, 3 to 5 mm. wide, was cut from the distended bladders and from those that had contracted to the point where folds just began to form. These zones were cut open along any meridian, thus converting them into ribbons, the length of which, measured just before imbedding, gave accurate results as to the length of the epithelium in the completely distended bladders. The ribbon-like strips were then cut into a convenient number of pieces, all of which were imbedded. Sections were cut perpendicular to the surface of the epithelium and parallel to the equator of the bladder. By projecting a section from each block of any particular zone, and measuring it with a wheel tracer, the length of the complete epithelium—i.e., the circumference of the circle which it forms in a complete section perpendicular to the long axis of the bladder at the equator—was calculated.

The lengths of the cells were then measured, in the direction parallel to the surface of the epithelium, in the same sections. It is evident that if there has been no slipping, the ratio of contracted to distended cells should be the same as that of contracted to distended epithelium. It must be borne in mind that the quantities here compared are linear quantities, and not areas, and that like dimensions, and not squares of like dimensions, are therefore compared.

Completely contracted bladders cannot be compared with greatly distended ones by this method, because of the impossibility of obtaining accurate information in regard to the length of the epithelium in the completely contracted bladder. When the folds of the epithelium first form, they are fairly regular

longitudinal, or rather meridional, folds. But on further contraction they become very irregular, probably due to the fact that the longitudinal muscle layer, contracting, carries with it the tunica propria that runs into these already formed folds. Figure 9 illustrates this point. It is a sketch of a portion of the epithelium of a completely contracted bladder in surface view. The bladder was found completely contracted in a freshly killed adult rabbit. Base and apex were cut off, and the bladder was

9

Fig. 9 Free-hand drawing of epithelial surface of completely contracted bladder (× 7.5). The vertical direction of the drawing is meridional with respect to the bladder. The figure shows that transverse sections may pass through the same fold three times.

cut open along a meridian. It was then spread out flat, with the epithelium uppermost. The corners were pinned down, and the specimen hardened in bichloride solution. A small square piece was cut out and sketched. The vertical direction of the drawing as here shown is longitudinal with respect to the bladder. It is easily seen that a section running at right angles to the long axis of the bladder might cross the same irregular longitudinal fold three times. Consequently, when the sections are studied, it is impossible to decide whether a given fold has been produced

by meridional or by equatorial contraction. To measure the length of the surface of the epithelium in sections across a completely contracted bladder is therefore useless. This was noticed during the preliminary work on this problem. The length of the contracted epithelium, multiplied by the amount of stretching observed in the cells, far more than accounted for the length of the distended epithelium. This was true even though all masses of epithelium within the main lumen, or all separate portions of the lumen not connected with the main lumen in the section, were left out of consideration.

The measurement of the length of the cells parallel to the surface of the epithelium was done with a micrometer eyepiece. It is essential that some method be employed which will result in representing all parts of a given circumference, and prevent undue choosing of certain types of cells that one comes to think of as typical. For example, one may take every cell in a given layer that has a nucleus and clearly defined boundaries, or every second or third cell of this description, according to the number of cells desired. The variation from cell to cell, in the same region, and from one part of the circumference to another, is very great. The largest cells may be six to ten times longer than the smallest. The first hundred cells in any given layer of a section, on the other hand, may be one and one-fourth times the size of the second hundred in the same section. But if the average of three hundred or more cells representing all parts of a given circumference is taken, and the measurements repeated in different sections from the same bladder, using the same method, the results will be nearly identical. So the method of taking every cell in a given layer, having a nucleus and clearly defined boundaries, and comparing the average size with that of cells similarly chosen from another bladder is an entirely reliable method of comparison. In the case of cells that have the shape of a parallelogram, the length of the side parallel to the surface of the epithelium is taken as the length of the cell.

The results obtained from several measurements of each epithelium, and about six thousand cell measurements, in material from the six rabbits, were as follows (table 1):

TABLE 1

Bladder no. 1 (distended)

Length of epithelium ..... 8 cm.
Length of cells:

		Average
1st (surface) layer	470 cells	51.4μ
2nd layer	385 cells	32.4μ
3rd layer	330 cells	18.8μ

Bladder no. 2 (completely contracted)

Length of epithelium ..... measurement impossible.
Length of cells:

1st layer	386 cells	27.6μ
2nd layer	275 cells	18.3μ
3rd layer	220 cells	10.1μ

Bladder no. 3 (partially distended)

Length of epithelium ..... 6.5 cm.
Length of cells:

1st layer	345 cells	46.2μ
2nd layer	385 cells	29.9μ
3rd layer	330 cells	18.9μ

Bladder no. 4 (completely contracted)

Length of epithelium ..... measurement impossible.
Length of cells:

1st layer	220 cells	27.7μ
2nd layer	220 cells	18.8μ
3rd layer	220 cells	9.6μ

Bladder no. 5 (contracted to beginning folding)

Length of epithelium ..... 3.8 cm.
Length of cells:

1st layer	393 cells	26.0μ
2nd layer	440 cells	17.6μ
3rd layer	330 cells	10.5μ

Bladder no. 6 (contracted to beginning folding)

Length of epithelium ..... 3.7 cm.
Length of cells:

1st layer	294 cells	25.9μ
2nd layer	385 cells	17.1μ
3rd layer	330 cells	10.8μ

Bladders no. 2 and no. 4 were completely contracted, so the length of epithelium is not entered for reasons previously stated. If we compare the contracted bladders no. 5 and no. 6 with the distended bladders no. 1 and no. 3, and find the ratio between the lengths of the epithelia and between the lengths of the cells of each layer, we obtain the following ratios (table 2):

TABLE 2

	RATIOS OF			
	No. 1 / No. 6	No. 1 / No. 5	No. 3 / No. 6	No. 3 / No. 5
Lengths of epithelia	2.16	2.10	1.75	1.71
Lengths of cells:				
1st layer	1.98	1.98	1.78	1.77
2nd layer	1.89	1.84	1.74	1.70
3rd layer	1.74	1.79	1.75	1.80

The significance of these results will now be discussed.

In the first place, it is evident that no great change in the length of the cells, measured parallel to the epithelial surface, occurs after folds have begun to form. The epithelia of bladders no. 2 and no. 4 were completely contracted and complexly folded. Nos. 5 and 6 were contracted to the point of beginning folding. Comparing the sizes of the cells, there is little difference. The surface cells (first layer) of nos. 2 and 4 are even slightly larger than those of nos. 5 and 6, probably because of the stretching at the crests of folds spoken of previously and illustrated in figure 7.

Secondly, the ratios between bladders no. 3 and no. 6 and between no. 3 and no. 5 show that there is no indication whatever of slipping of cells. The cells in bladder no. 3 have been stretched just as much as the epithelium. It must be borne in mind that 6.5 cm. represents the equatorial length of the epithelium of bladder no. 3 after fixing and dehydrating. In the fresh condition it measured 8.2 cm., so that the diameter at this point, or transverse axis, as it is sometimes called, was 2.6 cm. At this stage in distention the transverse axis is considerably shorter than the long axis, so that this bladder was well distended.

Thirdly, we notice that in comparing bladder no. 1 with nos. 5 and 6, the ratios indicating the stretching of the cells all fall short of the ratio indicating the stretching of the epithelium, and that they fall progressively shorter from the surface layer to the third layer. If bladder no. 3 is regarded as partly contracted, with reference to bladder no. 1 and the ratios determined, the same variation will again be established, so that bladder no. 1 is compared with three comparable bladders, with practically identical

results in every case. Still, the amount that the surface cells fall short is very small, and may be accounted for by the fact that as one measures the surface cells that have nuclei and clearly defined cell boundaries, fewer large cells than small cells conform to this standard. If the ratio is correct, it would mean that a piece of the surface layer that contained 198 cells in contraction contained 216 in distention, or that every twelfth cell in the surface layer of the distended bladder had crept in from a lower layer. The widest divergence is obtained by comparing the third layers of bladders 1 and 6. The cells of the third layer of no. 1 are 1.74 as long as those of no. 6, while epithelium no. 1 is 2.16 as long as that of no. 6. This means that a piece of the third layer that in contraction contained 174 cells in distention contained 216, or that every fifth cell, approximately, in the distended condition had entered the layer from another layer. If one imagines this, the greatest divergence found, occurring in each layer of five layered epithelium, it would be just sufficient to reduce the layers from five to four. The exact amount of rearrangement of cells indicated by taking into account all the ratios tabulated in comparing bladder no. 1 with bladders no. 5 and no. 6 would be a change from a four-layered epithelium in contraction to a 3.5-layered one in distention, or a five-layered one in contraction to a 4.4-layered one in distention. And this distention was a large one, for the circumference of the bladder before fixing was 10.4 cm. at the equator, which compares favorably with the greatest distentions seen in rabbits of twice the weight of this one, though more than a score of autopsies of such rabbits were taken note of.

Measurements similar to those above tabulated and discussed were also made on three bladders from adult rabbits. These rabbits were of the same litter, weighed a little more than 5 pounds each, and had been used for experiments in connection with studies on the development of bone.

The bladder of rabbit no. 8 was found contracted to the point where folding of the epithelium just begins. Rabbit no. 9 had a partly distended bladder. Rabbit no. 10 was played with for three-quarters of an hour, then anaesthetized with ether. The

abdomen was opened, and the bladder was found greatly distended. Its shape approached the spherical, as is the case in complete distention. Its equatorial circumference was 9.5 cm. before fixing. The technique employed was identical with that previously described.

The epithelia of these bladders had, on an average, nearly one layer of cells more than those of the previous series. Measurements were made of cells of three layers, and also of those basal cells situated between the tapering ends of cells which, though of the next higher level, also reach the basement membrane. The results were as follows (table 3):

TABLE 3

		Average
Bladder no. 8 (contracted to beginning folding)		
Length of epithelium		4.2 cm.
Length of cells:		
1st layer (surface)	477 cells	29.1μ
2nd layer	440 cells	20.0μ
3rd layer	330 cells	14.9μ
Basal cells	330 cells	9.7μ
Bladder no. 9 (partially distended)		
Length of epithelium		5.9 cm.
Length of cells:		
1st layer	440 cells	52.4μ
2nd layer	440 cells	28.6μ
3rd layer	316 cells	22.4μ
Basal cells	220 cells	12.5μ
Bladder no. 10 (distended)		
Length of epithelium		7.5 cm.
Length of cells:		
1st layer	427 cells	67.3μ
2nd layer	330 cells	33.7μ
3rd layer	330 cells	24.7μ
Basal cells	330 cells	14.4μ

A few ratios will suffice to point out the significance of these results (table 4):

In comparing the completely distended bladder with the contracted and with the partly distended one, therefore, the ratios obtained for the cells diminish in value from surface to base as

they did in the previous series. Comparing them with the ratios for the epithelia, the only striking irregularity and by far the largest of its kind in either series, is found in the ratio of the first layer cells of bladders 10 and 8. Here the stretching of the cells far more than accounts for the stretching of the epithelium. Comparing bladder no. 9 with bladder no. 8, we find a similar divergence. This means, no doubt, that bladder no. 8 had unusually small surface cells, while bladders no. 9 and no. 10 were comparable. The ratios in the middle column are therefore the important ones. The ratios in this column, taken together, indicate that the number of layers was diminished by 7.4 per cent

TABLE 4

	RATIOS OF		
	No. 10 / No. 8	No. 10 / No. 9	No. 9 / No. 8
Lengths of epithelia	1.78	1.27	1.40
Lengths of cells:			
1st layer	2.31	1.28	1.80
2nd layer	1.68	1.17	1.43
3rd layer	1.65	1.10	1.50
Basal cells	1.48	1.15	1.28

during distention, while those in the previous series indicated a maximum of 12.5 per cent diminution.

The possibility of cells 'slipping by one another' is therefore not eliminated. But it is evident that the decrease in layers, if present, is far less than the first glance at sections of contracted and distended bladders would suggest. Very slight tangency will increase the number of apparent layers when the cells are tall, as in contraction, whether the epithelium is folded or not. And this, with the thinness of the distended cells, accounts for the illusion.

Just what is meant by 'slipping by one another' is seldom mentioned. The only thing it could mean, so far as these studies suggest, may be shown by reference to figure 1. Beneath the only nucleus in the first layer is the junction of the two nucleated second-layer cells. If, on further distention, these two cells

should lose their attachment to each other, the cell just beneath would touch the surface cell, and the number of layers be reduced by one. The two second-layer cells might still maintain their attachment to the surface cell and to the cell between and beneath them, so that the integrity of the epithelium would not be endangered. Both histological study and the ratios above given suggest that such occurrences are more possible in the lower layers than in the surface layers, for in the latter the appearance of the cells suggests that they are bound very firmly to their lateral neighbors. Some of the basal cells are so imbedded in the basement membrane that this perhaps determines their position quite as much as attachment to the neighboring cells.

Turning again to figure 1, however, it is easy to see that a little more distention would cause the lower boundary of the nucleated surface cell, the connection between the second layer cells, and the upper boundary of the third layer cell to stain as one black line for a short distance. The interpretation of the microscopic picture would then become a matter of opinion. In the range of distentions above studied, which certainly equaled the range of normal physiological distention, there was little need for speculation, however, and the desired measurements could readily be made at a magnification of 950.

It is interesting to note that during the measurement of the first 6000 cells, which brought under observation several times that number, only one mitotic figure was observed, and this was in the layer next above the basal layer. During the measurement of over 4000 cells in the second series several mitotic figures were observed, and surface cells with two, three, or four nuclei were more frequent than in the first series.

Cuticular borders were not in evidence in these specimens, which were fixed in bichloride, and were in the final fixing solution within ten minutes after the death of the animals.

DISCUSSION

London, in 1881, studied bladder epithelium in contraction and distention in connection with the problem of resorption. He used bladders from dogs, and tried at first to distend them immediately after removal by forcing the fixative into the interior. This method he abandoned, because the prepared specimens showed a torn epithelium that he attributed to hardening of the epithelium by the fixative while distention still continued. He therefore distended the bladders by surrounding them with a negative pressure while ureters were left open at atmospheric pressure, and filled and surrounded them with fixative after the desired distention had been reached. He also used the method of fixing bladders with urine left inside to avoid contraction, but left the urine in these bladders twenty-four hours. His principal conclusions were: 1) that the thickness of the epithelium and all the layers of the bladder wall increases with age and size of the animal, and perhaps also with developed habits of retaining urine, as in the case of house dogs; 2) that the volume of the epithelial cells, or the entire epithelium, is the same in distention as in contraction, or that the thickness of the epithelium varies inversely as the area; 3) that the diminution of layers in distention is apparent, because of the diminution of layers of nuclei, but is not real if cell boundaries are taken into consideration. His closing statement that the epithelium possesses greater elasticity during contraction than during distention is very peculiar, unless we regard the epithelium as active rather than passive in contraction and distention, and ascribe to the cells changing conditions of tone. London's method of telling whether a section is tangent or not by noting whether the outline of the surface of the epithelium shifts on focusing is not infallible. The figures in this paper show that even the distended epithelium, when fixed, possesses irregularities, such as bulgings over nuclei in the surface layer, and other elevations and depressions. If a section, perpendicular to the general direction of the epithelial surface, includes one of these irregularities, the outline of the surface will shift on focusing. And in bladders fixed in contraction

each cell bulges into the lumen, presenting a spherical surface, so that the free surface outline will shift on focusing, regardless of whether the section is tangent to the general direction of the epithelium or not. Whether the thin lines London describes and interprets as cell boundaries of cut edges of cells were the same as the boundaries of cells without nuclei shown in the above figures is not certain. The above figures represent conditions in any one focal plane, and since the sections were 10μ thick, the narrow edges of cells shown could be traced to wider portions on focusing, or, in the lower layers, to the nucleated portion of the cell.

Dogiel ('90) studied the histology of the contracted bladder epithelium, especially of rodents, but also of dogs, cats, and man. He quotes Oberdieck as saying that in distention the surface cells flatten, while the deeper cells are displaced from their positions. He also cites Oberdieck's statement that bladder epithelium, in general, may be considered as three-layered epithelium, and in his own work distinguishes four layers—a surface layer whose cells are thick platelets, and whose shape, seen in surface view is irregularly polygonal; a second layer of irregular cylindrical or cubical cells, with long axis perpendicular to the epithelial surface; a third layer of somewhat cylindrical cells, the end nearer the epithelial surface being club-like and the opposite end tapering and reaching the basement membrane; a fourth layer of round, oval, or fusiform cells occupying the spaces between the narrowed lower ends of the third-layer cells. He made extensive studies of cells in macerated specimens. The surface cells are said to consist of an outer homogeneous third, and a deeper granular two-thirds, containing the nucleus or nuclei. The outer homogeneous portion (cuticular border) separates on maceration, and is claimed to be of a mucoid nature. The granular appearance of the cytoplasm is attributed to fibrillar protoplasmic network. Dogiel further claims to have demonstrated, and figures an extensive system of interlocking of the cells of the first two layers of epithelium, by projections of the second-layer cells fitting into depressions of the lower surface of the surface cells, and says that through these interlockings protoplasmic fibrils extend from

one cell to another. On this basis he founds his conclusion that these first two layers of cells permit of no rearrangement whatever and merely flatten in distention. This conclusion would be defensible only if an equally valid system of interlocking were demonstrated for the second-layer cells in their relations to one another, for, as shown in the discussion of the term 'slipping by one another' in connection with the cells of figure 1 above, it is shown that a cell need not lose all hold of its surroundings in order to part with the nearest neighbor in the same layer. The existence of the large projections of the second-layer cells that fit into depressions in the bottom of the first-layer cells is questioned by other observers, who used mainly dog material. Dogiel emphasized their prominence especially in material from small rodents, such as rats and mice. If they are sufficiently numerous in rabbit bladders to be of essential importance, they should be seen more frequently in sections, since a 10μ section takes in about half of a contracted second-layer cell. In the first series of bladders studied above, these projections were not seen at all. In the second series they were found occasionally. Wherever found, they were very definite structures. Whether fibrils extended from one cell to another could not be determined, because a definite cell boundary separated the cells. Dogiel describes these structures from macerated specimens. Harvey, in studying macerated preparations of bladder epithelium of dogs, did not corroborate these findings. He describes the cells as having a regular outline, and finds no structures or projections similar to those of Dogiel that could not be more readily considered products of maceration.

Eggeling ('01) studied the histology of the surface layer of bladder and ureter and reviewed the literature on the subject. He notes the recorded variability of a cuticular border in ureters from different animals and with various fixatives, which showed gradations from practical absence to virtual cornification of the entire surface layer. A description of the cuticular border, as demonstrated after alcohol-chloroform-acetic-acid fixation follows, and its significance in protecting the epithelium from contact with urine and in preventing or reducing resorption is dis-

cussed. The existence of canals in the epithelium, described by Lendorf, is not corroborated, and the evidence of secretory activity of the surface cells is considered doubtful.

To each of these articles a large bibliography is appended, but the subjects treated deal mainly with the histology for any static condition—studies on channels in epithelia, studies on goblet cells, etc.—and do not relate to distention.

Harvey, in 1909, published an interesting article on variations in the wall of the bladder and ureter in contraction and distention. He used dogs, and distended the excised bladders by forcing in Zenker's fluid. Staining was done with hematoxylin and congo-red. Cell boundaries are described as distinct only in the contracted bladder and in the surface layer of the distended. In the other layers of the distended bladder they are "discontinuous or in fragments, as though the cytoplasm of adjacent cells had fused in places, or distention has made the membranes so thin as to be invisible." The nuclei are depended upon in this inquiry into the relations that cells assume in contraction and distention, as in the earlier work of Herzog. In regard to bladder epithelium, the following conclusions are reached 1) that the distended epithelium is one-sixth the thickness of the contracted; 2) that the number of layers of nuclei is decreased 50 per cent, approximately, in distention; 3) that there may be, in addition to the stretching of cells, a slight displacement of the cells from their relative position, hence, an actual diminution of the number of layers.

The diminution in the number of layers of nuclei is interesting. The first conclusion above tabulated, together with the work of London, which showed that the volume of the epithelium in distention is the same as that in contraction, show that the surface of the epithelium distended by Harvey increased six times. A line perpendicular to the epithelium at any point would have a sixth as many chances of piercing nuclei in the distended bladder as it would have in the contracted. And a plane, passing entirely through the contracted and distended bladders at comparable points would have 2.4—the square root of six—times the number of chances of encountering nuclei in the contracted bladder that

it would have in the distended, if the nuclei remained unchanged in size. But they do not. Measurement of 110 nuclei of contracted bladder no. 6 of the above series, and 110 nuclei of the distended bladder no. 1 showed that while the epithelium had been stretched along the equator to 2.1 times its former length, the nuclei had been stretched to 1.14 times their former diameter parallel with this direction. If a stretching of 2.1 times increased the diameter by 0.14, a stretching of 2.44, as in Harvey's work, would probably increase it to 1.16 times its former length. This would counteract the effect of increasing distances between the centers of nuclei, so that a plane having a chance of passing through 244 nuclei in the contracted bladder would pass through 116 in the distended. This amounts to a 52.4 per cent decrease, or very nearly the percentage decrease established by Harvey in counting the layers of nuclei in a given focal plane.

SUMMARY

The results of this investigation, so far as the principal problem stated at the beginning is concerned, may be summarized as follows:

1. Moderate physiological distention of the rabbit's bladder is reached without any evidence that the cells of the epithelium are displaced from their relative positions, that is, without any decrease in the number of layers.

2. Very great physiological distention of the rabbit's bladder probably results in a slight decrease in the average number of layers. This decrease is scarcely demonstrable without making cell measurements, and these show that it does not amount to more than 12.5 per cent.

3. Histological evidence and measurements confirm the idea that the cells of the epithelium are bound to one another more firmly in the first and second layer than in the third or deeper layers.

It is obvious that the conclusive data cover only the range of distention and contraction that lies between beginning folding of the epithelium and maximum physiological distention. Whether the cells form more layers in complete contraction is not known.

All that is shown is that they do not contract further after folds have begun to form.

In conclusion, I wish to acknowledge my indebtedness to Dr. E. R. Clark, under whose direction this investigation was carried out.

BIBLIOGRAPHY

Dogiel, A. S. 1890 Zur Frage über das Epithel der Harnblase. Archiv für microscopische Anatomie, Bd. 35, S. 389–407.

Eggeling, H. 1901–02 Ueber die Deckzellen im Epithel von Ureter und Harnblase. Anat. Anzeiger, Bd. 20, S. 116–123.

Harvey, R. W. 1909 Variations with distention in the wall and epithelium of the bladder and ureter. Anat. Rec., vol. 3, pp. 296–307.

Lewis and Stöhr 1913 A textbook of histology. Blakiston's, Philadelphia, second edition, p. 324.

Lee, A. B. 1913 Microtomist's vade mecum, seventh edition. P. Blakiston's Sons & Co., Philadelphia.

London, B. 1881 Das Blasenepithel bei verschiedenen Füllungszuständen der Blase. Arch. für Physiol., S. 317.

Mallory and Wright 1913 Pathological technique, fifth edition. W. B. Saunders & Co., Philadelphia.

Resumen por el autor, W. N. Hess,
Cornell University, Ithaca.

Tráqueas en los órganos luminosos de algunos Lampíridos comunes.

Comparando los principales troncos traqueales y sus ramificaciones mas importantes en los segmentos abdominales de algunos de nuestros mas comunes lampíridos, el autor ha encontrado que la disposición de estas ramificaciones es semejante en cada segmento abdominal excepto en el noveno, y que no existen nuevas ramificaciones en adición a los principales troncos traqueales. La principal diferencia consiste en el excesivo tamaño de los troncos principales y sus ramificaciones en los segmentos en que están colocados los órganos luminosos. Aun cuando no nacen nuevas ramificaciones en los troncos principales, las que existen son mas gruesas y parecen dividirse mucho mas frecuentemente en ramillas mas y mas finas, que las que existen en otros segmentos abdominales, con el fín de aportar mayor cantidad de aire a los órganos luminosos.

En los machos de nuestros lampíridos mas luminosos algunos de los conectivos longitudinales de las tráqueas situadas en los segmentos que contienen los órganos productores de luz, en vez de unir entre si los troncos traqueales de estos segmentos, se han dividido y ramificado considerablemente, conduciendo directamente el aire a los órganos luminosos.

Translation by José F. Nonidez
Cornell Medical College, New York

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, NOVEMBER 15

TRACHEATION OF THE LIGHT-ORGANS OF SOME COMMON LAMPYRIDAE[1]

WALTER N. HESS

TEN FIGURES

The light-organs of all the luminous fireflies that have been described occupy a part, or the entire ventral portion, of one or more abdominal segments. In a large per cent of luminous males the organs occupy the entire ventral portions of the sixth and seventh abdominal segments, while not infrequently the organs of the females are much smaller and restricted to a small area in the sixth segment. In our luminous larvae the organs are usually paired and lie in the ventro-lateral portion of the eighth abdominal segment.

The structure of the light-organs in all insects that have been studied, whether in the larva, pupa, or adult, appears to be very similar. They have always been found to consist of an inner, non-photogenic layer called the reflector and an outer luminous, or photogenic, layer (figs. 1 and 2).

The author is indebted to Dr. William A. Riley, under whose direction this study was made, for his helpful suggestions and criticisms.

Numerous workers have studied the structure of the mature light-organs, and in this connection many have figured the arrangement of the tracheal end-cells within the organ itself. Yet so far as any detailed study of the arrangement of the main tracheal trunks and branches that supply the light-organs is concerned, nothing seems to have been done except a brief work by Geipel ('15). He figured, in a semidiagrammatic way, their arrangement in the male of Luciola africana. According to his

[1] Contribution from the Entomological Laboratory of Cornell University.

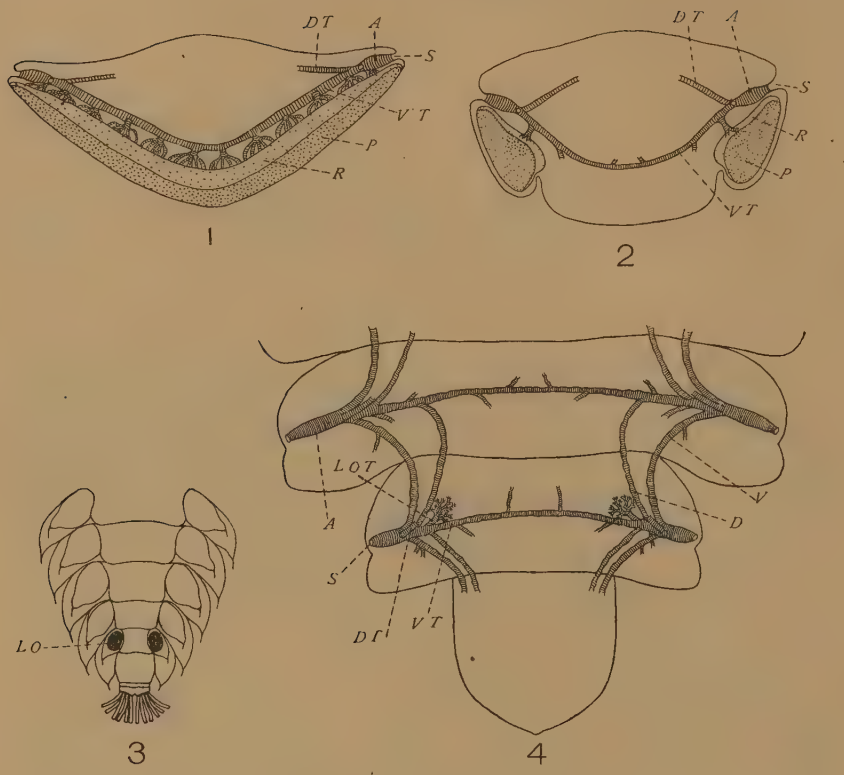


Fig. 1 Photinus scintillans, male, diagrammatic drawing to represent the two layers of the light-organ and the general arrangement of the larger trachea. *A*, ampulla; *D T*, dorsal tracheal trunk; *P*, photogenic layer; *R*, reflector layer; *S*, spiracle; *V T*, ventral tracheal trunk.

Fig. 2 Photurus pennsylvanica, larva, diagrammatic drawing to show the two layers of the light-organ, and the arrangement of the trachea. *A*, ampulla; *D T*, dorsal tracheal trunk; *P*, photogenic layer; *R*, reflector layer; *S*, spiracle; *V T*, ventral tracheal trunk.

Fig. 3 Photurus pennsylvanica, larva, ventral view of abdomen. The dark elliptical areas which are located on the eighth abdominal segment represent the larval light-organs, *L O*.

Fig. 4 Photurus pennsylvanica, larva, dorsal view of the principal trachea of the seventh and eighth abdominal segments. *A*, ampulla; *D*, dorsal longitudinal tracheal connective; *D T*, dorsal tracheal trunk; *L O T*, light-organ trachea; *S*, spiracle; *V*, ventral longitudinal tracheal connective. All tracheae shown with small branches supply the light-organ.

description, the main tracheal branches arise irregularly from the ventral transverse tracheal connectives.

In order better to understand the general relation of the tracheal trunks and their branches which supply oxygen to the light-organs of the adult and larva, a diagrammatic drawing was made of a cross-section through the seventh segment of a male of Photinus scintillans (fig. 1), and through the eighth abdominal segment of the larva of Photurus pennsylvanica (fig. 2). Both figures readily show the dorsal and ventral tracheal trunks which separate from the ampulla near the spiracle. In the case of the adult, the ventral trunk gives off numerous branches, which soon divide, sending their little tubes into the light-organ. These tubes pass through the reflector layer into the photogenic layer where they end in numerous tracheoles. The light-organs of the larva, however, are each supplied by one large tracheal branch, which leaves the ventral trunk near the ampulla. As it enters the light-organ it divides profusely, sending its branches throughout the region of the photogenic layer (*P*).

In the larva of Photurus pennsylvanica (fig. 3, *L O*) the light-organs appear externally as two small oval areas on the ventro-lateral sides of the eighth abdominal segment.

Since it was found that the arrangement of all the main tracheal branches in the different abdominal segments of the larva, except the ninth, are similar to those shown in the seventh and eighth segments (fig. 4), the trachea of only these two segments were figured. As can be readily seen by a comparison of the tracheation in these two abdominal segments, the branches arising from the transverse trunk in each segment are similar and they are bilaterally arranged. The trachea which supply oxygen to the light-organs in segment eight are simply enlarged branches which arise from the ventral transverse tracheal connective (*V T*) and correspond to similar branches which arise from the ventral transverse tracheal connective in segment seven. The branch from the ventral tracheal trunk in segment eight which supplies the light-organ (*L O T*) is shown with many of its branches.

The adult female of Photurus pennsylvanica, which is one of our largest native luminous fireflies, has the light-organs restricted

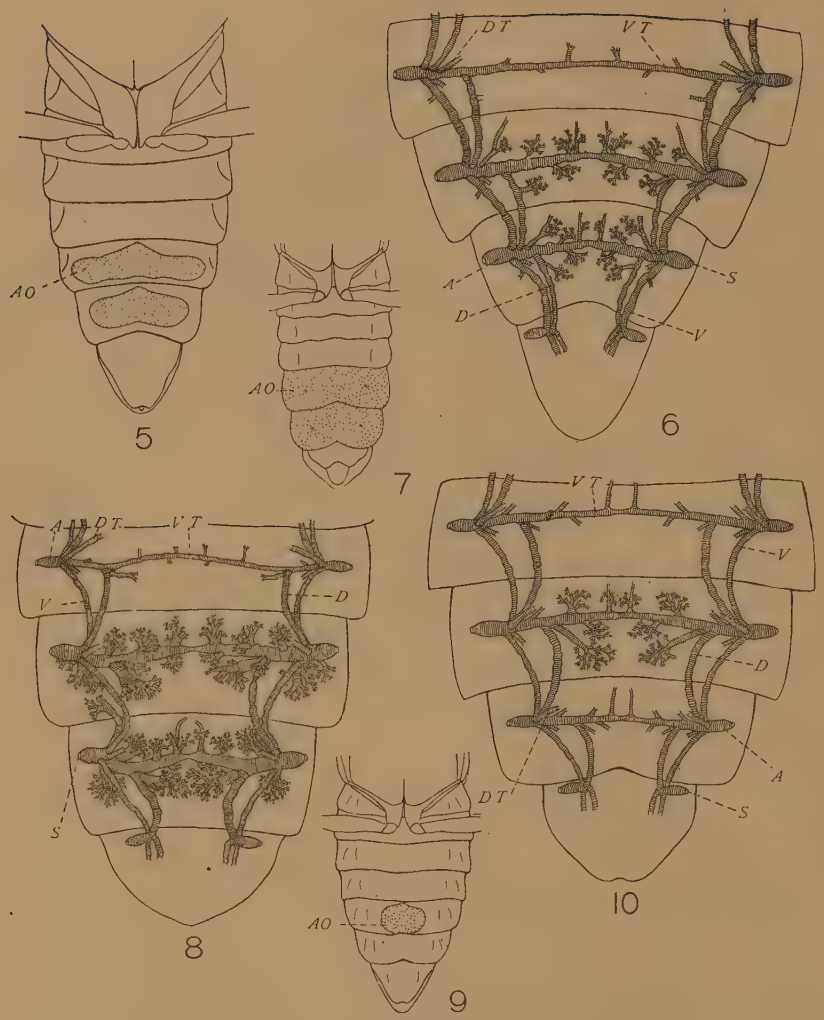


Fig. 5 Photorus pennsylvanica, female, ventral view of abdomen. The stippled areas on the sixth and seventh abdominal segments represent the adult light-organ (*A O*).

Fig. 6 Photurus pennsylvanica, female, dorsal view of the principal trachea of the fifth, sixth, and seventh abdominal segments. For labels see figure 4.

Fig. 7 Photinus scintillans, male, ventral view of abdomen. The stippled areas on the sixth and seventh abdominal segments represent the adult light-organ (*A O*).

Fig. 8 Photinus scintillans, male, dorsal view of the principal trachea of the fifth, sixth, and seventh abdominal segments. For labels see figure 4.

Fig. 9 Photinus scintillans, female, ventral view of abdomen. The shaded area on the sixth abdominal segment represents the adult light-organ (*A O*).

Fig. 10 Photinus scintillans, female, dorsal view of principal trachea of the fifth, sixth, and seventh abdominal segments. For labels see figure 4.

to an area covering about two-thirds of the sternites of the sixth and seventh abdominal segments (fig. 5, *A O*). These organs are supplied with branches that arise from the ventral transverse tracheal connectives of the sixth and seventh abdominal segments, which are shown with their many branches (fig. 6). The arrangement of the tracheal branches in both segments is the same, and these branches correspond to similar trunks found in segment five, as well as to those in the other anterior abdominal segments. These trunks are much smaller than they are in the male, due to the fact that the organs are smaller, and hence they do not require as large an air supply.

The tracheation of the light-organs of the male Photurus pennsylvanica was not figured, as it was found to be similar to that of the male of Photinus scintillans, which will be discussed.

The adult male of Photinus scintillans, like most of our other luminous males, has its light-organs extensively developed so that they cover the entire sternites of the sixth and seventh abdominal segments (fig. 7, *A O*). The main tracheal trunks of this insect in the sixth and seventh abdominal segments were found to be of immense size (fig. 8), in order to supply the large amount of air that is needed by these light-organs. A brief description of these enlarged trunks will readily show the extreme of tracheal specialization for the supply of air to the light-organs of fireflies. The arrangement of the tracheal branches which arise from the ventral transverse tracheal connective of segment six is practically identical with the arrangement of the branches which arise from the corresponding trunk in segment five. The chief difference is that they are larger and the finer branches appear much more numerous. The arrangement of the branches in the sixth abdominal segment correspond very closely to those in the seventh abdominal segment. The longitudinal tracheal connectives between these two segments have a tendency to become modified into branches which supply the tissues of the light-organs, but no new branches were found to arise from the transverse tracheal connective of either segment. In the specimen that was figured the ventral longitudinal tracheal connective (*V*) between segments six and seven, on the left side, is not

continuous, but has divided near the middle, and has become much branched for the purpose of supplying air to the light-organs. On the right side this connective, in the specimen which was drawn, was found to be very narrow, and at one point it showed evidence of a tendency to divide. The ventral transverse tracheal connectives in segments six and seven are very large from the spiracles to the center of the body. Here they become the same size as corresponding trachea in the other abdominal segments. In no case are accessory branches found to arise from the ventral transverse tracheal connectives, but the branches frequently divided and subdivided into numerous smaller branches, as soon as, or soon after, they arose from the main transverse trunks. A small amount of air for the light-organs evidently enters the spiracles of the fifth and eighth abdominal segments as certain of the longitudinal tracheal connectives leading from these segments to those of the light-organs are larger than in the other segments.

The female of Photinus scintillans, which has a very small light-organ in the sixth abdominal segment (fig. 9, *A O*) naturally has a reduced air supply. The main tracheal trunks of the sixth abdominal segment are somewhat enlarged (fig. 10), but they are small in comparison with the corresponding trunks in the male. As is also true of the other fireflies studied, no new tracheal branches are found to arise from the ventral transverse tracheal connectives, but these branches divide into numerous smaller branches in the region of the light-organs, for the purpose of supplying, with a large amount of air, the tissues of this structure.

By a comparison of the main tracheal trunks and their principal branches in the abdominal segments of some of our common luminous fireflies, it was found that the arrangement of these branches was similar in each abdominal segment except the ninth, and that no new branches were added. The chief difference was found in the excessive size of the main tracheal trunks and their branches in the segments where the light-organs were located. While no new branches arose from the main tracheal trunks, those present appeared to divide much more profusely into finer and

finer branches than the corresponding trunks in the other abdominal segments, for the purpose of supplying a large amount of air to the light-organs. In the male fireflies of our most luminous species some of the longitudinal tracheal connectives in the segments containing the light-organs were not found connecting the tracheal trunks of these segments, but instead they were greatly branched supplying air directly to the light-organs.

PROCEEDINGS OF THE AMERICAN SOCIETY OF ZOÖLOGISTS

EIGHTEENTH ANNUAL MEETING

The American Society of Zoölogists held its Eighteenth Annual Meeting at the University of Chicago in conjunction with Section F. of the American Association for the Advancement of Science and in association with other biological societies, December 28, 29 and 30, 1920.

The officers for the year were:

President: Gilman A. Drew.
Vice-President: Caswell Grave.
Secretary-Treasurer: W. C. Allee.
Local Committee: H. H. Newman, Chairman, C. R. Moore and A. W. Bellamy.
Executive Committee: R. P. Bigelow, H. V. Wilson, M. M. Metcalf, George Lefevre and C. M. Child.

In the absence of the Secretary, H. V. Neal was elected Secretary *pro tem.*

BUSINESS TRANSACTED

The principal business session was held Thursday morning, December 30. The business was completed at an adjourned session held at noon of the same day.

Business from the Advisory Board

The Advisory Board authorized in 1919 and organized as follows: To serve one year, V. E. Shelford and C. C. Nutting; to serve two years, J. T. Patterson and Robert Chambers, Jr.; to serve three years, M. M. Metcalf and G. N. Calkins; to serve four years, W. E. Castle and F. R. Lillie (Chairman), recommended approval of a continuation of conferences between the Secretaries of the various Biological Societies.

The board also recommended approval of the following memorandum from the Ecological Society of America:

Report on the Preservation of Wild Life

The Ecological Society of America's committee on the preservation of natural conditions, while unable to deal with problems concerning wild life not in reserves, continually encounters the fact that individual species are menaced with extinction by agricultural encroachments. Two of these menaces are:

1. Clean-culture (roadside mowing and burning) as distinguished from roadside and streamside shrubbery and bird and original life preservation.

Birds are decreasing for lack of nesting sites, on account of destruction of breeding conditions. Entomologists and some agriculturists maintain that this condition is necessary to agriculture. Bird men insist that birds are also essential. It is known that a few states encourage roadside shrubbery while several require roadside mowing. The practice in the various parts of the United States and Canada should be ascertained. The effect of different procedures should be determined. The areas in which specially destructive and drastic measures such as burning for insect pests are necessary should be clearly defined and limited and the public informed as to the dangers of such burning.

2. Upland marshes are important as sponges storing water and letting it out slowly during dry seasons, thus controlling floods. Such marshes are gradually being drained and the flood menace is increasing every year.

The only way to save these natural resources and at the same time, the swamp faunas, especially the birds, is to utilize the swamps for agriculture. To this end several water-culture experiment stations should be established. For the present there should be one, perhaps at Cornell University, to deal with the upland marsh problems. There should be another in connection with Okefinokee swamp and one in connection with the coastal swamps of New Jersey. In addition to frogs, fish, and birds, a number of plants are good for food, etc.; e.g., cattail flour and cattail paper have recently been tried with success. Swamp potatoes, the corms of arrowhead, and seeds, roots, and stalks of our native lotus served as food for the American aborigines and pioneers. Hedrick (Science, 40: 611), Claussen (Sci. Mo., 9: 179), and Needham and Lloyd (Life of Inland Waters) have discussed these questions and suggested or advocated the improvement and culture of aquatic plants.

It is the belief of the committee that all organizations in any way interested should combine efforts for the investigation of these questions.

V. E. Shelford,
University of Illinois, Chairman.

The Society unanimously approved both recommendations.

Business from the Executive Committee

The following change in the Constitution having been approved and published as required by Article VI was unanimously passed:

Article II, add Section 4.

Foreign Zoölogists, not members of this Society, may be elected Honorary Fellows upon unanimous recommendation of the Executive Committee by a majority vote of the members present at any meeting of the Society. Honorary Fellows shall not be required to pay dues.

By-Law 5 was ordered amended to read:

It shall be the policy of the Society to hold its annual meetings in both Eastern and Central territory and the distribution of meetings between the two territories shall be determined in general on the basis of the representation of Eastern and Western members in the Society.

The Constitution and By-Laws as amended will be found on pages 233–236 of this *Journal*.

Election of Members

The Executive Committee recommended the following persons for election to membership in the Society:

1. Chapman, Royal N., B.A., M.A. (Minnesota), Ph.D. (Cornell), Assistant Professor of Animal Biology; Assistant Entomologist, *Experiment Station, University of Minnesota, Division of Entomology, University Farm, St. Paul, Minn.*
2. Dawson, James Arthur, A.B. (Dalhousie), A. M., Ph.D. (Yale), Professor of Biology, Dalhousie University, *Halifax, Nova Scotia, Canada.*
3. Dunn, Leslie Clarence, S.B. (Dartmouth), S.M., Sc.D. (Harvard), Poultry Biologist, *Connecticut Agricultural Experiment Station, Storrs, Conn.*
4. Duporte, Ernest Melville, M.Sc., B.S.A. (McGill), Instructor in Zoölogy and Entomology, Macdonald College, *Macdonald College P. O., Province Quebec, Canada.*
5. Fraser, Charles McLean, A.B., A.M. (Toronto), Ph.D. (Iowa), Professor of Zoölogy, University of British Columbia and Director of Marine Biological Station, Nanaimo, *University of B. C., Vancouver, B. C.*, or *Biological Station, Nanaimo, B. C.*
6. Goldsmith, William Marion, B.Pd., A.B., A.M., Ph.D. (Indiana), Professor of Biology, Southwestern College, *Winfield, Kansas.*
7. Grier, Norman McDowell, S. B., A.M., Ph.D. (Pittsburgh), Professor of Biology, Washington and Jefferson College, *Washington, Pa.*
8. Hecht, Selig, Ph.D. (Harvard), Assistant Professor of Physiology, *Creighton Medical College, Omaha, Neb.*
9. Hess, Walter N., A.B. (Oberlin), A.M., Ph.D. (Cornell), Professor of Biology, De Pauw University, *Greencastle, Indiana.*
10. Jewell, Minna E., A.B. (Colorado College), A.M., Ph.D. (Illinois), Assistant Professor of Zoölogy, *Milwaukee-Downer College, Milwaukee, Wisconsin.*

11. Job, Thesle T., A.B., A.M., Ph.D., Associate Professor of Anatomy, Loyola University School of Medicine, *706 S. Lincoln St., Chicago, Ill.*
12. Kudo, Rokusaburo, D.Ag.Sc. (Tokio), Instructor in Zoölogy, *University of Illinois, Urbana, Illinois.*
13. Lillie, Ralph S., A.B. (Toronto), Ph.D. (Chicago), Biologist, Nela Research Laboratories, Department of Pure Science, *Nela Research Laboratories, Nela Park, Cleveland, Ohio.*
14. Lippincott, William Adams, A.B. (Illinois College), S.B. (Iowa State), S.M., Ph.D. (Wisconsin), Professor of Poultry Husbandry and Poultry Husbandman, Kansas State Agriculture College, *Manhattan, Kansas.*
15. McCulloch, Irene, Ph.D. (California), Professor of Biology, Sophie Newcomb College, *New Orleans, La.*
16. May, Henry G., S.B. (Rochester), Ph.D. (Illinois), Professor of Bacteriology Rhode Island State College and Chief, Division of Animal Breeding and Pathology, Agr. Exp. Station, *Kingston, R. I.*
17. Muttkowski, Richard Anthony, A.B., A.M., Ph.D. (Wisconsin), Assistant Professor of Zoölogy and Entomology, University of Idaho, *Moscow, Idaho.*
18. Olmsted, J. M. D., Ph.D. (Harvard), Instructor in Physiology, Toronto University, *Toronto, Canada.*
19. Snyder, Thomas Elliott, A.B. (Columbia), M.F. (Yale), Ph.D. (George Washington), Specialist in Forest Entomology, *Bureau of Entomology, U. S. Department of Agriculture, Washington, D. C.*
20. Swingle, Wilbur Willis, A.B., A.M. (Kansas), Ph.D. (Princeton), Instructor in Biology, Sheffield Scientific School, Yale University, *Osborn Zoölogical Laboratory, New Haven, Conn.*
21. Taylor, Charles Vincent, Ph.D. (California), Assistant Professor of Zoölogy, University of California, *East Hall, University of California, Berkeley, Calif.*
22. Turner, Clarence Lester, A.B., A.M. (Ohio Wesleyan), Ph.D. (Wisconsin), Professor of Zoölogy, Beloit College, *Beloit, Wisconsin.*
23. Weese, Asa Orrin, A.B. (Minnesota), A.M. (Illinois), Professor of Biology, University of New Mexico, Secretary, Ecological Society of America, *Albuquerque, N. Mex.*

These nominees were unanimously elected.

The Journal of Morphology

The Committee on Publication appointed at St. Louis submitted the report given below to the Executive Committee. Acting by the authority given it at St. Louis, the Executive Committee approved the action of the Committee and the report gives the basis for the coöperation between the Society and The Wistar Institute in publishing the *Journal of Morphology.*

THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY
Philadelphia, Pa.

April 5th, 1920.

Dear Dr. Metcalf: In order to make perfectly clear the relation of the American Society of Zoologists to The Wistar Institute and the *Journal of Morphology*, I wish to confirm the impression that you have that the Society is to elect the Managing Editor and Associate Editors of the *Journal of Morphology*, and to make such arrangements as may seem desirable for the scientific control of this journal.

Yours very truly,

(Signed) M. J. GREENMAN.

Dr. M. M. Metcalf
Philadelphia, Pa.

To the Executive Committee of The American Society of Zoologists:

Sirs:—In accordance with your instructions, your Committee, consisting of W. E. Castle, Caswell Grave and Maynard M. Metcalf, "appointed

(1) to initiate a scientific policy concerning the *Journal of Morphology;*

(2) to nominate an Editorial Board;

(3) to consult with the Advisory Board of The Wistar Institute concerning both the proposed policy and the editorial nominations;

(4) to refer the recommendations for final action to the Executive Committee in 1920 and thereafter through the Executive Committee to the Society at its annual meeting," first consulted with each other by correspondence and in person, and afterward met with the Advisory Board of The Wistar Institute at its annual meeting, April 4th to 6th, in Philadelphia.

There was full and frank discussion at this meeting of the matters involved.

Your Committee being unanimous in its desire to nominate Prof. C. E. McClung to be Managing Editor of the *Journal of Morphology*, and having learned that such nomination would be particularly pleasing to the Director of The Wistar Institute and its Board of Advisors, obtained Prof. McClung's consent to the nomination and then asked him to meet with us in consultation upon the other items submitted to us for recommendation.

Dr. Greenman, Director of The Wistar Institute, was also further consulted in regard to all the matters mentioned in this report and there has been complete agreement of your Committee and of Prof. McClung and Dr. Greenman in the following recommendations:

I. That there be elected a Managing Editor of the *Journal of Morphology* to serve for a period of five years and that he be eligible to reelection at the expiration of his period of service.

Your Committee feels that there is no objection to long tenure of the office of Managing Editor of the *Journal* and that this is desirable.

II. That there be elected nine Associate Editors of the *Journal of Morphology*, three to serve until January 1st, 1922; three to serve until January 1st, 1923: and three to serve until January 1st, 1924.

That beginning with the annual meeting of the Society at the end of the year, 1921, and annually thereafter, there be elected by the Society upon nomination by the same method as is provided for the nomination of other officers, three Associate Editors to serve for three years to take the places of the three retiring Asso-

ciate Editors. That before making nomination of such Associate Editors, the Nominating Committee shall consult the Board of Editors of the *Journal of Morphology* and also the Director of The Wistar Institute and through him the Board of Advisors of this Institute.

This is suggested as a courtesy to the Institute, not as a matter of necessity, for the final authority for the election of the Editors of this *Journal* will lie with the Society.

That a retiring Associate Editor shall not be eligible to reelection until after the expiration of one year subsequent to his retirement.

III. That the three Associate Editors elected to serve until January 1st, 1924, be constituted for the year 1921 a consulting committee to visit The Wistar Institute upon its invitation and at its expense, to familiarize themselves with the work of the Institute and to take from the Society to the Institute and receive from the Institute for the Society, suggestions as to the relations between the Society and the Institute and as to opportunities for coöperation not only in matters of publication, but in other matters as well in which the coöperation of the Institute and the Society may promote the interests of Zoölogy.

The Institute has suggested the election of such a Committee and has urged that the Society through this Committee make with freedom and frankness suggestions that may enhance the usefulness of the Society and of the Institute in their common service to Zoölogical science.

The Society should, in this connection, realize the policy of the Institute to limit for the present the field of its activity with a view to greater effectiveness through concentration of effort.

The Institute further desires from year to year, such a Consulting Committee in order that there may soon come to be in the Society a considerable number of its members who shall understand the purposes, plans and methods of operation of the Institute with a view to most effective coöperation between the two organizations.

Your present Committee recommend that this Consulting Committee be the three Associate Editors elected to serve for three years, so that they may have opportunity to enter upon their service as Editors with fuller understanding of the institution with which they will be coöperating in their editorial duties.

We further recommend that a similar Committee be appointed for the year 1922, and annually for each year thereafter and that this Committee consist annually of the three newly elected Associate Editors.

Your Committee desires to express its sense of the great value of such mutual understanding of the Society and the Institute of one another's spirit, purposes plans and methods, this sense being based upon our own informing and delightful experience as guests of the Institute for these two days.

IV. That the Board of Editors make annual report to the Society upon the *Journal of Morphology* and any matters of publication that they may wish to include.

V. That the Consulting Committee or any of its members, if they desire to do so, may report any year to the Society any suggestions or recommendations growing out of their visit to and consultations with The Wistar Institute.

VI. That Prof. C. E. McClung be elected Managing Editor of the *Journal of Morphology*.

VII. That Associate Editors of the *Journal of Morphology* be elected as follows:

1. To serve until January 1st, 1922
   Prof. Gary N. Calkins
   Prof. J. S. Kingsley
   Prof. Wm. Patton
2. To serve until January 1st, 1923
   Prof. E. G. Conklin
   Prof. M. F. Guyer
   Prof. W. M. Wheeler
3. To serve until January 1st, 1924
   Prof. C. A. Kofoid
   Prof. F. R. Lillie
   Prof. J. T. Patterson

VIII. That matters of Editorial Policy and method, not covered by the present report, be left to the Board of Editors composed of the Managing Editor and the Associate Editors, subject, of course, to any action by the Society.

It may be well to state that no fundamental changes in the character or conduct of the *Journal of Morphology* is contemplated and that it is planned to continue publishing in the *Anatomical Record* the Proceedings of the Society.

IX. That your present Committee be discharged at such time as you shall have received and have acted upon this our report.

Your Committee cannot refrain from expressing its deep sense of the generosity and the fine spirit of coöperation shown by The Wistar Institute. They are giving to our Society the unrestricted management and use of the *Journal of Morphology* which is owned by the Institute. Their only desire clearly is to increase their own efficiency and the effectiveness of the Society of Zoölogists in promoting biological research through coöperation. They not only wish to establish at once coöperation in publication, but they invite suggestion from time to time as to coöperation in other ways; the relation they suggest between the two organizations is as unique as it is generous and your Committee believes that it is another instance of statesman-like vision such as is shown in the Institute's plan, already realized, to distribute its publications more widely than any scientific serial publications have heretofore been distributed.

The only worthy thanks we can suggest from the Society to the Institute lie in accepting the proffer of coöperation and joining our efforts in effective service to zoölogical research.

We append to this report a letter from Dr. Greenman which makes clear the generous confidence of The Wistar Institute in the Society of Zoölogists.

Philadelphia, Pa.
April 5th, 1920.

(Signed) W. E. Castle,
Caswell Grave,
Maynard M. Metcalf.

C. E. McClung reported to the Society concerning the present status of the *Journal of Morphology* and on motion by Dr. McClung the Society recommended that the subscription price of the *Journal of Morphology* be increased from $9.00 to $12.00 per volume.

Report of the Secretary

During the year 1920, two members of the Society have been removed by death, E. L. Michael and George D. Allen; three have resigned and six members have been dropped for non payment of dues, leaving at present, before the election of new members, 305 members of the Society.

During the year, the American Association for the Advancement of Science, recognizing the high standard of the American Society of Zoölogists in choosing their membership, agreed to recognize election to membership in the Society as a certification of eligibility to being enrolled as a Fellow in the Association. In the future, all members elected will be immediately nominated as Fellows in the Association.

The Secretary called the attention of members who enter nominations or membership that it is very important that a complete list of the nominees' publications be filed with page references and in addition that a short biographical sketch be furnished similar to that found in American Men of Science.

The report was ordered to be placed on file.

Treasurer's report for 1920.

Receipts to December 24, 1920

Balance on hand at last report		$1,204.30
68 $5.00 dues	$340.00	
201 7.00 dues	1,407.00	
17 11.50 dues	195.50	
6 irregular sums from members	23.30	
Refund from Wistar Institute	13.50	
Credit at Wistar Institute	2.00	
Interest on Savings deposits	43.69	
Uncorrected error by bank clerk	10.00	
		2,034.99
Total receipts		$3,239.29

Expenditures to December 24, 1920

To Wistar Institute for subscriptions:		
January 1	$367.00	
February 5	6.00	
July 8	1,590.00	
December 1	159.50	
Total		$2,122.50

Expenses of office of Secretary-Treasurer:			
Printing			
Tickets for Zoölogy dinner at St. Louis	$0.50		
Programs, envelopes and bills and mailing same	44.78		
Announcements	13.50		
Total for printing		$58.78	
Stenography, typewriting and office help		24.25	
Postage		19.22	
Exchange advanced on Canadian check		0.80	
Telephone and telegrams		1.75	
Express			
Programs to St. Louis	0.68		
Typewriter and records to and from Woods Hole	6.00		
Total express		6.68	
Record book for minutes of the Society		3.00	
Expenses of Secretary-Treasurer to St. Louis		41.97	
Total expenses of office of Secretary-Treasurer		$156.45	
Total expenditures			$2,278.95
Cash on hand December 24, 1920			
Savings account		$898.56	
Checking account		61.78	
Total cash on hand		$960.34	
Total cash on hand plus expenditures			$3,239.29
Bills due, payable January 1, 1921			
Wistar Institute for subscriptions	$73.50		
Correction, error of bank clerk	10.00		
Total		$83.50	
Probable balance, January 1, 1921			
Balance, December 24, 1920	$960.30		
Approximate interest on savings	13.50		
Total		$973.80	
Bills payable, January 1, 1921		83.50	
Probable balance, January 1, 1921			$890.30
Balance, January 1, 1920		$809.59	
Net gain for the year			$80.71

In addition there are 38 members in arrears for dues aggregating 61 years and amounting to $399.50, $32.50 more than last year.

The Auditing Committee, consisting of Bennet M. Allen and N. E. McIndoo, reported that they had audited the above accounts and found them correct.

The reports of the Treasurer and the Auditing Committee were received and ordered placed on file.

Election of officers

The Nominating Committee (See Art. 3, Sec. 6), composed of D. H. Tennent, Charles Zeleny and R. A. Budington, report the following nominations:

President, C. A. Kofoid.

Vice-President, A. L. Treadwell.

Member Executive Committee, to serve five years, G. A. Drew.

Member of Division of Biology and Agriculture, National Research Council, to serve three years, William Patten.

Members of the Council of A. A. A. S., C. C. Nutting, W. C. Allee.

There were no other nominations and the officers, as presented by the Nominating Committee, were duly elected.

The Executive Committee announced the appointment of C. A. Kofoid and D. H. Tennent to fill the places on the Advisory Board made vacant by the retirement of V. E. Shelford and C. C. Nutting.

Resolutions

The Society adopted the following resolutions:

Regarding duty free importation of scientific materials

The American Society of Zoölogists representing the zoölogical interests of the country, especially from the standpoint of research and instruction in our American Colleges and Universities, views with much concern the proposal made in the bill H. R. 7785 which provides for an increase of 20 per cent in the duty on scientific instruments and an increase of 30 per cent on scientific glassware and in addition repeals section 573 of the tariff act of October 3, 1913, which allows for the duty free importation of such materials by educational institutions.

In view of the fact that the great mass of research in pure science is still carried on by men in our colleges and universities, an increase in the cost of scientific apparatus and equipment is especially to be deplored since even under the present arrangement of low duties and duty free import privileges, the funds at the disposal of our educational institutions are inadequate to provide for the most efficient teaching equipment or to allow for the most effective prosecution of research:

Therefore be it resolved: That the American Society of Zoölogists, assembled in annual session, call the attention of Congress to the burden imposed upon the prosecution of educational and research work by the proposed repeal of the privilege of duty free importation of scientific apparatus, chemicals and glassware by educational institutions and respectfully request the continuance of this privilege in proposed tariff legislation.

The American Society of Zoölogists also requests the restoration of the privilege of the duty free importation of single copies of scientific books in the English language by members of recognized educational and scientific institutions.

That copies of these resolutions be forwarded to the Congressional Committees concerned, the National Academy of Sciences, the National Research Council and to the Executive Committee of the American Association for the Advancement of Science, and given other proper publicity as the Executive Committee of the American Society of Zoölogists shall direct.

Concerning the preservation of wild life

Whereas: The Ecological Society of America is engaged in attempting to secure the reservation of natural areas, i.e., reserves including the original flora and fauna in an undisturbed state, for research present and future. A standing committee has been listing and describing such areas reserved and desirable for reservation, during several years past. The Society is now entering on a plan to unite the various groups interested in primeval areas, namely: 1. Investigators in biology, geography, history and art. 2. Sportsmen through their interest in game sanctuaries. 3. Ornithologists through their interest in bird refuges. 4. Wild flower lovers through their interest in primeval areas as seeding centers and preserves.

The purpose of such union of effort will be to secure the preservation of natural areas in state parks, forest preserves, etc., and to secure the creation of more such parks and forest preserves.

Whereas: The number of primeval preserves, especially in the Eastern states, is wholly inadequate for either present or future research purposes and areas from which such preserves may be created are rapidly being destroyed.

Be it resolved that: The American Society of Zoölogists indorses the efforts of the Ecological Society of America to secure reserves for research purposes and directs its Secretary to forward a copy of this resolution to the Division of Biology and Agriculture of the National Research Council.

And further resolved that: The President of the Society be directed to appoint a delegate to the Parks Conference to be held in Des Moines, Iowa, January 10, 11 and 12, 1921, said delegate to represent the Society in the interest of reserves of primeval conditions for zoölogical research.

The incoming President, C. A. Kofoid, appointed V. E. Shelford to be the delegate of the Society to this conference.

Sessions for the presentation and discussion of papers

During the sessions for the presentation and discussion of papers, 54 papers were read in full, 13 of which were followed by discussion. The sessions were occupied as follows:

TUESDAY, DECEMBER 28

Room M11, Harper Library

9:00 A.M. Session for reading and discussion of papers on General Physiology.
2:00 P.M. Session for reading and discussion of papers on Embryology.

Ida Noyes Hall

8:00 P.M. Address of the retiring Vice-President of Section F. of the American Association for the Advancement of Science, W. M. Wheeler, Harvard University. *Subject:* The organization of research. The address was followed by the Biological Smoker.

WEDNESDAY, DECEMBER 29

Section A. Room M11, Harper Library

9:00 A.M. Session for reading and discussion of papers on Cytology and General Zoölogy.

Section B. Room 14, Zoölogy Building

Papers on Comparative Anatomy and Parasitology.

Section A. Room 14, Zoölogy Building

2:00 P.M. Session for reading and discussion of papers on Evolution and Genetics.

Section B. M 11, Harper Library

Joint session with the Ecological Society of America for reading and discussion of papers dealing with Ecology and Zoögeography.

THURSDAY, DECEMBER 30

Room M 11, Harper Library

9:00 A.M. Business meeting of the Society.
10:00 A.M. Symposium on Fertilization.

LIST OF TITLES

The following titles, contributed for the program, have been grouped and arranged in accordance with rules accepted by the Society.

Papers marked by an asterisk were read by title

COMPARATIVE AND GENERAL PHYSIOLOGY

1. The sensory physiology of glochidia and the mechanism of encystment. Leslie B. Arey, Northwestern University Medical School.
2. Observations of the feeding habits of oysters. (Lantern.) Thurlow C. Nelson, Rutgers College and New Jersey State Board of Shell Fisheries.
3. Responses of the de-eyed larvae of *Amblystoma tigrinum* to solid bodies. Albert Kuntz and José Zozava, St. Louis University School of Medicine.
4. The auditory sense of the honey bee. N. E. McIndoo, Bureau of Entomology, Washington, D. C.
5. The active role of oxygen in the development of fertilization eggs of rotifers. (Lantern.) David D. Whitney, University of Nebraska.
6. An experimental study of the tarsal chemoreceptors of two nymphalid butterflies. Dwight E. Minnich, University of Minnesota.

*7. The relative stimulating efficiency of continuous and intermittent light in the tachina fly, *Archytas aterrima.* William L. Dolley, Jr., Randolph-Macon College.

8. Relation of body form to spiral movement. A. A. Schaeffer, University of Tennessee.

*9. Poisonous spiders. A. M. Reese, West Virginia University.

*10. The minor pedicellariae of two species of Pacific Coast starfishes. C. O. Esterly, Occidental College.

*11. Some points concerning adaptation. W. J. Crozier, Rutgers College.

*12. Reactions to light in the larvae of the Ascidian, *Amaroucium.* S. O. Mast, The Johns Hopkins University.

13. Control of morphological polarity by means of the electric current. E. J. Lund, University of Minnesota.

14. Resistance of fish to different concentrations of salt. R. T. Young, University of North Dakota.

15. Sterilization by means of spermatoxins. M. F. Guyer, University of Wisconsin.

*16. The olfactory reactions of *Amblystoma tigrinum.* J. S. Nicholas, Yale University. (Introduced by Henry Laurens.)

EMBRYOLOGY

*17. The shrinkage of embryos in the processes preparatory to sectioning. Bradley M. Patten and Rees Philpott, Western Reserve University Medical School.

18. The experimental production of twins in the starfish, *Patiria miniata:* with a discussion of the causes of twinning in general. H. H. Newman, University of Chicago.

19. Bifurcation in the embryos of tubifex. Paul S. Welch, University of Michigan.
20. Relative sizes of pig embryos. Mary T. Harmon, Kansas State Agricultural College.
21. The effects of transplantation of the several parts of the adult hypophysis into tadpoles in *Rana pipiens*. Bennet M. Allen, University of Kansas.
22. Lipolytic effects of egg secretion. O. C. Glaser, Amherst College.
*23. Proliferation and differentiation in the central nervous system of *Amblystoma*. G. E. Coghill, Department of Anatomy, University of Kansas.
24. Sex gland transplantation and the modifying effect in rats and guinea pigs. Carl R. Moore, University of Chicago.
25. The development of connective tissue in the chick embryo. George A. Baitsell, Osborn Zoölogical Laboratory, Yale University.
26. Some habits of *Cirratulus* in relation to fertilization. John W. Scott, University of Wyoming.
*27. Homoplastic and heteroplastic endocrine transplants. W. W. Swingle, Osborn Zoölogical Laboratory, Yale University. (Introduced by R. G. Harrison.)
28. Is the initiatory effect of egg secretion specific? Alvalyn E. Woodward, Amherst College. (Introduced by O. C. Glaser.)
28a. R. A. Budington appealed for support for Professor Van der Stricht and his *Archives of Biologie* which can be given through the purchase of lantern slides made from Van der Stricht's preparations showing fertilization in *Nereis*.

CYTOLOGY

29. Cytology of *Anisolabis maritima*. (Lantern.) S. I. Kornhauser, Denison University.
30. Protoplasmic viscosity changes in the dividing egg of *Cumingia*. L. V. Heilbrunn, University of Michigan.
31. The structure and division of *Trichomonas muris* (Hartman). D. H. Wenrich, University of Pennsylvania.
*32. A comparison of a Limax Amoebae with tissue culture cells. M. J. Hogue, School of Hygiene and Public Health, Johns Hopkins University.
*33. Unusual tetrads and their bearing on the problem of crossing-over. W. R. B. Robertson, University of Kansas.
34. Spermatogenesis in Cestodes. R. T. Young, University of North Dakota.
35. Conjugation and fission-rate in *Arcella vulgaris* (Ehrenberg). Hansforth M. MacCurdy, Alma College.
*36. The occurrence of precocious and abortive maturation phenomena in female larvae of *Rana catesbeiana*. W. W. Swingle, Osborn Zoölogical Laboratory, Yale University. (Introduced by R. G. Harrison.)
*37. Peculiar chromosomal phenomena in a Homopteran. Franz Schrader, Bryn Mawr College. (Introduced by L. V. Heilbrunn.)
38. The early history of the germ cells in the brook lamprey, *Entosphenus wilderi* (Gage), up to and including the period of sex differentiation. Peter Okkelberg, University of Michigan. (Introduced by Paul S. Welch.)

GENERAL ZOÖLOGY

*39. Something of the life history of a fresh water medusa. F. Payne, Indiana University.

COMPARATIVE ANATOMY

40. The history of the vertebrate mouth. H. V. Neal, Tufts College.
41. The origin of the cerebral hemispheres. C. Judson Herrick, University of Chicago.
*42. The integumental glands of *Alligator mississippiensis*. A. M. Reese, West Virginia University.
43. The order, time and rate of ossification of the vertebrate skeleton. R. M. Strong, Loyola University School of Medicine.
44. Primary neuromeres and vertebrate head segmentation. H. W. Stunkard, New York University. (Introduced by J. S. Kingsley.)
45. Studies on lymph nodes: 1. Structure, as shown by deposited ink granules. Thesle T. Job, Loyola University School of Medicine. (Introduced by R. M. Strong.)
46. The topography of the cloaca of the male *Necturus* in relation to cloacal glands. Alden B. Dawson, Loyola University School of Medicine. (Introduced by R. M. Strong.)

PARASITOLOGY

*47. Measurements of *Trypanosoma diemyctili* from different hosts and their relation to specific identification, heredity and environment. R. W. Hegner, School of Hygiene and Public Health, Johns Hopkins University.
48. Notes on the development and distribution of *Oncicola canis* (Kaup 1919). H. J. Van Cleave, University of Illinois.
49. *Dermatobia hominis*. Thomas Byrd Magath, Mayo Clinic.
*50. The development of the Japanese blood-fluke, *Schistosoma japonicum* Katsurada, in its final host. W. W. Cort, School of Hygiene and Public Health, Johns Hopkins University.
51. The course of migration of *Ascaris* larvae from the intestine to the lungs. B. H. Ransom and Eloise B. Cram, U. S. Bureau of Animal Industry.
*52. Life history of a new Limax Amoeba. M. J. Hogue, School of Hygiene and Public Health, Johns Hopkins University.
53. Correlation of the life-cycle of a parasite with the metamorphosis of its host. Nadine Nowlin, University of Kansas.
*54. Observations on the occurrence and life history of *Cephalobium microbivorum* (Cobb). James E. Ackert, Kansas State Agricultural College.
55. Studies on the sheep stomach worm, *Haemonchus contortus*. John E. Guberlet, Oklahoma Agricultural Experiment Station.
56. Acanthocephala from the American eel. H. J. Van Cleave, University of Illinois.

EVOLUTION AND GENETICS

57. The production of mosaic males from fertilized eggs in Hymenoptera. P. W. Whiting, St. Stephen's College.

58. The direction and frequency of mutation in a series of multiple allelomorphs. Charles Zeleny, University of Illinois.
59. On the relation of stale sperm to fertility and sex in ring-doves. Oscar Riddle and Ellinor H. Bêhre, Department of Experimental Evolution, Carnegie Institution of Washington.
60. Genetic analysis of low crossover stock produced by selection. J. A. Detlefsen, College of Agriculture, University of Illinois.
61. A note on the inheritance of polydactylism in cattle. E. Roberts, College of Agriculture, University of Illinois.
62. Selection in Cladocera. (Lantern.) Arthur M. Banta, Station for Experimental Evolution.
63. A study of the character and mode of origin of eighteen mutations in the X-chromosome of *Drosophila*. H. J. Muller, University of Texas, and E. Altenburg, Rice Institute.
64. Further studies on inheritance of color in the turkey. W. R. B. Robertson, University of Kansas.
65. Experiments with typhoid agglutinins in rabbits. M. F. Guyer and E. A. Smith, University of Wisconsin.
66. Flat-fish with unusual pigmented areas. (Lantern.) Arthur M. Banta, Station for Experimental Evolution.
67. A new type of sex-linked lethal in Drosophila. David H. Thompson, University of Illinois. (Introduced by Charles Zeleny.)
*68. The course of evolution in the king snakes. Frank N. Blanchard, University of Michigan. (Introduced by Paul S. Welch.)

Joint meeting with Ecological Society of America

ECOLOGY AND ZOOGEOGRAPHY

Contributed by members of both Societies except as noted

These papers were read at a joint session with the Ecological Society of America. Unless otherwise noted, papers are by members of both Societies.

69. The circulation of water in the Bay of Fundy, and its bearing on the distribution of the fauna. James W. Mavor, Union College.
70. The geographical distribution of the Anura and their Opalinid parasites. M. M. Metcalf. (A. S. Z.)
71. Distribution of fishes in Wisconsin lakes. A. S. Pearse, University of Wisconsin.
72. Data on the distribution of fresh-sponges in North America. Frank Smith, University of Illinois.
73. Quantitative and chemical studies of the plankton of Lake Mendota. (Lantern.) C. Juday, Wisconsin Geological and Natural History Survey.
74. Hydrogen ion concentration of natural waters and the importance of its measurement. (Lantern.) V. E. Shelford, University of Illinois and Illinois Natural History Survey.
75. An analysis of the spawning habits of Chaetopleura apiculata. B. H. Grave, Wabash College. (A. S. Z.)
76. South America and its intercontinental land connections as indicated by a survey of the geographical distribution of the Anura and their Opalinid parasites. M. M. Metcalf. (A. S. Z.)

77. Relation of temperature and tides to the quantity and distribution of oyster larvae. (Lantern.) E. P. Churchill, University of South Dakota.
78. Some reactions of the jelly-fish, Aequorea. A. O. Weese and M. T. Townsend. (E. S. A.)
79. Longevity of the Trogoderma tarsale larvae without food. J. E. Wodsedalek, University of Idaho.
80. The problem of the local distribution of Thiasiapillus imbricatus (Lamarck). Harold S. Colton, University of Pennsylvania.

SYMPOSIUM ON FERTILIZATION

81. Some phases in the evolution of sexual reproduction in the Protozoa. (30 minutes.) C. A. Kofoid, University of California.
82. The initial event in fertilization. (20 minutes.) F. R. Lillie, University of Chicago.
83. The susceptibility of the inseminated egg to hypotonic sea-water; A contribution to the analysis of the fertilization reaction. (20 minutes.) E. E. Just, Howard University.
84. Fertilization and egg secretions. (20 minutes.) O. C. Glaser, Amherst College.
*85. Chromosomes and fertilization. (20 minutes.) C. E. McClung, University of Pennsylvania.
86. Chromatic material in hybridization. (20 minutes.) D. H. Tennent, Bryn Mawr College.

Papers received too late to be listed on the regular program.

†87. Allelomorphism in Bruchus. J. K. Breitenbecher, University of Oklahoma. (Introduced by A. Richards.)
†88. A new method of ingestion exemplified in the Ciliate, Frontonia. William M. Goldsmith, Southwestern College. (Introduced by S. O. Mast.)
†89. Copper: Its occurrence and rôle in insects and other animals. Richard A. Muttkowski, University of Idaho. (Introduced by J. E. Wodsedalek.)
90. The postembryonic development of the compound eye of Drosophila melanogaster. Joseph Krafka, Jr., University of Georgia. (Introduced by Charles Zeleny.)
91. The sex element in the flash of the firefly. C. R. Fountain, Mercer University. (Introduced by R. A. Budington.)

Nos. 90 and 91 were called and read by special vote of the Society.

EXHIBITS

Room 24, Zoology Building

1. A larva of Dermatobia hominis. Thomas Byrd Magath, Mayo Clinic.
2. Dove embryo "monsters" and abnormalities obtained by altered pressures of oxygen. Oscar Riddle, Station for Experimental Evolution.
3. A small, continuous acting centrifuge for the recovery of minute aquatic organisms. C. Juday, Wisconsin Geological and Natural History Survey.
4. Rabbits showing inherited eye-defects induced by fowl serum sensitized against the crystalline lens of the rabbit. M. F. Guyer and E. A. Smith, University of Wisconsin.

† Not called.

5. Specimens of Trogoderma tarsale larvae undergoing starvation. J. E. Wodsedalek, University of Idaho.
6. Twelve quarto, colored plates of a monograph on the Dinoflagellata. C. A. Kofoid, University of California.
7. A series of 2-mm. human embryos. George W. Bartelmez, University of Chicago.
8. Quadruplets in Armadilli. H. H. Newman, University of Chicago.
9. Apparatus for measuring light intensity. V. E. Shelford, University of Illinois.
10. Breeding experiments with tropical fish. A. W. Bellamy, University of Chicago.

ABSTRACTS

COMPARATIVE AND GENERAL PHYSIOLOGY

1. The sensory physiology of glochidia and the mechanism of encystment. LESLIE B. AREY, Northwestern University Medical School.

Fresh water mussels pass through a larval stage known as the glochidium. Metamorphosis is contingent upon a temporary ectoparasitic existence upon the gills, or other soft parts, of appropriate fish-hosts. In this investigation the range of sensory-motor activities of the minute, bivalved glochidia has been tested, especially for the purpose of ascertaining the nature of the stimuli which lead to their attachment on the host and induce the latter to initiate cyst proliferation.

There are two great groups of glochidia: some species possess valves equipped with hooks; the majority, however, are hookless. It is the existing belief that hooked glochidia attach to hosts because of a closure of the valves resulting from a tactile, or contact stimulus; hookless forms, on the contrary, are denied a delicate tactile sensibility, and, it is said, the effective stimulus is a chemical one, derived from exuding blood.

Detailed experimentation proves not only that glochidia of both types respond actively to touch, the receptive organs being the hair cells of the mantle, but that this tactile response is entirely adequate to insure attachment. Although glochidia react to a wide range of chemical agents, and to some in great dilution, chemical excitation through minute gill haemorrhages, or otherwise, is not responsible for the usual attachment of either hooked or hookless glochidia to fishes. An interpretation based upon mechanical contact satisfied the facts, and this tactile responsiveness is adequate to produce the infections obtained both experimentally and in nature.

The formation of a cyst about an attached glochidium is likewise basicly a mechanical response—the overgrowth of an abrading foreign body by host tissue. Experiment indicates that the glochidium itself does not induce the response through chemical stimulation; moreover, encystment has been successfully imitated by affixing to excised gill filaments minute metallic clips, the size of glochidia or smaller. There is, however, a superimposed regulatory factor which normally prevents the formation of too bulky a cyst; this influence is lost to a filament after its excision.

2. Observations of the feeding habits of oysters. (*Lantern.*) THURLOW C. NELSON, Rutgers College and New Jersey State Board of Shellfisheries.

The present paper is a preliminary report on series of studies of food and feeding of oysters, begun in March, 1919, at floating laboratory

for oyster investigations of New Jersey Experiment Station, at Little Egg Harbor, New Jersey. Experiments carried on in open waters with oysters on bottom surrounded by natural conditions. Recording apparatus above the surface registered all movements of oyster shells, and at each complete opening or closure, samples of water surrounding the oyster were tested for density, temperature, turbidity, and food content.

Results of experiments show: Average of 20 hours activity per day. Sixty per cent of hours of inactivity during night. Seventy-four per cent of closures took place during ebb and low tide, of which only twelve per cent due to lowering of density below normal limits of toleration. Sixty-three per cent of openings occurred during flood and high tide. Oysters accustomed to density fluctuations from 1014 to 1018 did not open to feed in water below 1008. Feeding continued in waters bearing as high as 0.2 gm., dry weight, of suspended matter per litre. Mud and discarded slime strings ejected at frequent intervals by sudden partial closure of shell, contractions of adductor muscle becoming at times almost rhythmical. No correlation found between feeding periods and abundance of food. Oysters found to vary rate of filtration independent of temperature. Stereoptican illustrations show apparatus used and curves made by oysters during feeding.

3. Responses of the de-eyed larvae of Amblystoma tigrinum to solid bodies. Albert Kuntz and José Zozaya, St. Louis University School of Medicine.

De-eyed larvae of Amblystoma tigrinum respond in a characteristic manner to a solid body which approaches the head or lateral surface of the anterior portion of the trunk in the water although actual contact does not occur. This response is probably the result of stimuli received by receptors in the integument.

4. The auditory sense of the honeybee. N. E. McIndoo, Bureau of Entomology, Washington, D. C.

Beekeepers are agreed that bees can hear, yet they cannot prove it and critics still contend that it has never been experimentally proven that any insect can hear; nevertheless, within the last few years some good experimental results have been obtained.

The special sound-producing apparatus of the honeybee consists of the membranes lying between the axillaries at the bases of the front wings. Muscles, lying in the thorax and attached to these axillaries, contact and relax very quickly, thereby causing the axillaries to vibrate; consequently the above membranes are caused to vibrate rapidly, thus producing the piping, teeting, or squealing noise, commonly heard when a bee is squeezed.

Up-to-date five so-called auditory organs have been found. Judging from their anatomy, the pore plates, Forel flasks, pit pegs and Johnson's organ, all located in the antennae, do not seem to be well fitted to act as sound receptors; but the chordotonal organs, lying in

the tibiae seem to be better adapted for this purpose. The Johnson's organ consists of the peculiarly modified articular membrane between the second and third antennal segments and of many sense cells whose fibers unite with peculiar knobs extending inwardly from the articular membrane. The chordotonal organs are quite complicated, resembling those in certain Orthoptera.

The sound-producing apparatus and Johnson's organ in the honey-bee, and also a minor detail in the histology of the pore plates, are here described for the first time.

5. The active rôle of oxygen in the development of fertilization eggs of rotifers. (*Lantern.*) DAVID D. WHITNEY, University of Nebraska.

The fertilized eggs of the rotifer, Brachionus bakeri, have thinner surrounding envelopes than many other species of rotifers. If freshly produced eggs are placed in water through which a stream of air is passing nearly all of them will segment. At the end of 52 hours over 90 per cent of them will contain embryos ranging from the 2 cell stage to the young that are ready to emerge. Very few of these eggs hatch because the envelopes are so firm and tenacious that the young are unable to break through them. If these eggs are not aerated they do not develop.

It was found that if these newly-laid fertilized eggs were sealed up in vials together with large numbers of micro-organisms and the whole mass allowed to decompose 3–8 days, they would immediately begin to develop when aerated. The young would develop and break out of the egg membranes in less than 18 hours.

The decomposition of the organic matter effects the egg membranes in such a manner that they not only are more permeable to oxygen but also are readily broken open by the young rotifer when it is ready to emerge.

6. An experimental study of the tarsal chemoreceptors of two nymphalid butterflies. DWIGHT E. MINNICH, University of Minnesota.

The nymphalid butterflies, Pyrameis atalanta Linn. and Vanessa antiopa Linn., respond to chemical stimulation by uncoiling the proboscis. By means of this response, it has been possible to locate and to study some hitherto unknown organs of chemical sense in these butterflies. These organs are located in the tarsi of the four walking legs, and, while their exact distribution has not been determined, experiments show that they are present at least in that portion of the tarsus comprising the distal end of the proximal segment and the four distal segments. The organs in question are contact chemoreceptors, that is, they are stimulated through intimate contact with the source of stimulating material.

In both Pyrameis and Vanessa, the tarsal chemoreceptors distinguish applejuice, which is one of the natural foods of these animals, from distilled water, although the organs are sensitive to both substances. Additional experiments on Pyrameis showed that it is also able to dis-

tinguish a 1M saccharose solution from distilled water, and such solutions as 1M HCl, M/600 quinine sulphate, and 1M NaCl from either distilled water or 1M saccharose. One very important function of the tarsal chemoreceptors, therefore, appears to be in connection with food substances and water. Not only are the animals enabled to detect the presence and exact location of such substances in the substrate, but they are also able to make some discrimination as to their nature.

7. The relative stimulating efficiency of continuous and intermittent light in the tachina fly, Archytas aterrima. WILLIAM L. DOLLEY, JR., Randolph-Macon College, Ashland, Va.

Ten photopositive flies were tested in intermittent light of an illumination of 35 m.c. at the following flash-frequencies, 2, 5, 10, 20, 30, 40, and 50 per second. Six insects were each tested in the same flash-frequencies as above in the following illuminations, 3 m.c. and 100 m.c. In an illumination of 35 m.c. intermittent light of certain flash-frequencies has a greater stimulating effect than continuous light of equal illumination, the maximum being at a flash-frequency of approximately 15 per second. As the flash-frequency is raised above 15 per second the stimulating efficiency of intermittent light decreases until it becomes equal to that of continuous light at slightly over 50 per second. As the flash-frequency is lowered below 15 per second the stimulating efficiency of intermittent light decreases until it becomes equal to that of continuous light at about 5 per second and less than that of continuous light at 2 per second. In an illumination of 100 m.c. the maximum stimulating efficiency of intermittent light is at a flash-frequency of approximately 20 per second, while in 3 m.c. it is at a flash-frequency of between 5 and 15 per second.

The results of these experiments consequently are in harmony with those previously reported for Vanessa antiopa in showing that the stimulating efficiency of intermittent light depends upon the flash-frequency, i.e., it may be greater, equal to, or less than that of continuous light. They also indicate that the flash-frequency at which intermittent light has a maximum stimulating efficiency varies with the illumination.

8. Relation of body form to spiral movement. A. A. SCHAEFFER, University of Tennessee.

The discovery that, as far as known, all motile organisms move in some form of spiral path when the orienting senses are not functioning has raised the question whether there is any relation between the shape and position of the body and the form of the path. Considered as a problem in engineering it is found that some one-celled organisms with spiral shaped bodies swim in a spiral path that conforms with the form of the body while in others the spiral path turns in a direction that is the opposite of that of the form of the body; the former swim along the path of least resistance, the latter against it. Again the position of the body, that is, whether the long axis is perpendicular to or in the plane of the

surface on which the organism moves, does not materially influence the spirality of the path. The only organism readily fulfilling these conditions is man. Numerous records of men and women while swimming under experimental conditions show definite spiral paths which are similar in general to the paths made by persons while walking.

9. Poisonous Spiders. A. M. Reese, West Virginia University.

For many years there has been a difference of opinion in regard to the poisonous characteristics of spiders, especially in regard to the so-called "black" or "black widow" spider, *Latrodectes mactans.*

Comstock and other authorities, in spite of numerous apparently authentic cases that have been reported, have maintained that this spider is at least not deadly, and is possibly not even dangerous to man.

In 1915 V. L. Kellogg reported a case in which a man in California was dangerously affected by the bite of a spider that was caught and identified as *L. mactans.*

The most recent case that has come to the attention of the writer, reported to him by the sister of the victim and by one of the attending physicians, was a Mr. L— of Oklahoma, a strong, healthy man of thirty-eight years. As in nearly all of the cases previously reported, he was bitten on the penis while using an outcloset. The resulting symptoms were as in other similar cases; violent, spasmotic pains that refused to yield to ordinary narcotic treatment; restlessness, etc. He died about thirty-two hours after being bitten, in spite of the efforts of three physicians.

10. The minor pedicellariae of two species of Pacific Coast starfishes. C. O. Esterly, Occidental College.

In addition to pedicellariae of the type well known as crossed minor, the starfishes Pisaster capitatus and P. ochraceus have two kinds of small pedicellariae, not yet described, in which the jaws do not cross. The straight form called "elbows" are found along with the crossed minors around the bases of the spines on the aboral surface. The other small straight pedicellariae are located almost exclusively at the bases of the gill filaments and for that reason are called papular.

The three sorts of minor pedicellariae differ in behavior as well as in structure. The activities of the crossed minors in Pisaster are similar to those described for Orthasterias (Asterias) forreri by Jennings. The elbows remain closed under varied conditions. They do not open even when the animal is fairly bristling, with the crossed minors standing up beyond the ends of the spines and the jaws gaping widely. If the elbows are touched they sink down into the soft flesh until invisible, and if the integument or a tuft of gills is stroked with a bristle the elbows nearby disappear. Occasionally an elbow pedicellaria opens without any apparent cause, and it remains open if touched or if the flesh or a tuft of gills is tapped lightly with a bristle or small glass rod. It is very unusual, however, to find any of the elbows open.

The papular pedicellariae can be observed when the gills are expanded well. When the animal is undisturbed most of the jaws are

nearly closed but one here and there will open and close slowly. One pedicellaria alone will open if the outside of the jaw is touched but many on a tuft of gills open when the surrounding flesh is tapped or when the ends of the gill filaments are lightly swept. An open pair of jaws snaps shut if touched on the inside, but not if the point stimulated is on the outside of one of the jaws. A pedicellaria swings around and over toward a point near it that is touched and opens during the movement or after it is completed.

The crossed minors are known to be most efficient organs, but the actual usefulness of the elbow and papular pedicellariae may be questioned. The functions of both the latter are problematical.

11. Some points concerning adaptation. W. J. Crozier, Rutgers College.

1. *Oxyhaemoglobin in Ascaris.* The observations of several writers is confirmed that oxyhaemoglobin is present in the tissue spaces of A. lumbricoides, commonly regarded as an anerobic organism. There is some reason for the opinion oxygen carried in this way may be important for fertilization of the eggs and for their earliest development. The alkalinity of the body juice of Ascaris is about equal to that of other animals, pH 7.6.

2. *On the growth of the fins in teleosts.* In Epinephelus various linear dimensions of the body, including the radial lengths of the several fins, are directly proportional to the length of the individual. The *area* of each of the fins is proportional to the square of the body length. Each fin consequently grows, during post larval life, at a constant rate. The different sizes of the several fins, as related to their separate functions, must therefore be due to some property of the organization of the animal as a whole, resulting in the establishment of differently sized primordia previous to the onset of the constant growth-rate—and not to adaptive differential deviations from this fundamental rule of growth in fishes during post-larval life.

3. *The periodic swarming of Odontosyllis enopla.* The hour of swarming of this luminous annelid, following a lunar periodicity, changes during the successive months of the breeding season (May to November), in such a manner as to be nicely adjusted throughout to the time of nightfall and of moonrise.

4. *Persisting rhythm of light-production in balanoglossids.* Ptychodera and Glossobalanus may maintain for some eight days a clear-cut diurnal rhythm of light-production while remaining continually in the dark. The reciprocal experiment could not be made, for even at night the expulsion of luminous materials is promptly inhibited by illumination.

5. *Multiplication by fission in a balanoglossid.* Ptychodera was in some species shown by Willey to occur in two forms, differing in the length of the gill series. These forms are demonstrated to result from the general occurrence of the habit of autotomous division, a normal method of multiplication in this species, especially during summer months.

6. *Photic sensitivity of the skin of Ogilbia.* O. verrellii Garman, found under stones in the shore zone at Bermuda, is nearly related to certain blind fishes of Cuban caves. According to Eigenmann, these cave fishes probably exhibit integumentary photosensitivity, O. verrillii is remarkably sensitive to light (negatively phototropic), although its eyes are quite small, and its skin is photosensitive—the second case of its kind known among many tested marine genera. This condition is of prime importance for the understanding of the preadaptation of the eyeless cave fishes in this respect.

12. Reactions to light in the larvae of the Ascidian, Amaroucium. S. O. Mast, The Johns Hopkins University.

The larvae of Amaroucium are much like a tadpole in shape but they have only one eye and this is located at the posterior end of the body near the base of the tail.

When they emerge from the colonies they are strongly photo-positive, after a few moments they become photo-negative and remain so until they become attached and begin to metamorphose.

If the light is rapidly reduced resting specimens, both positive and negative, respond by becoming active and active specimens respond by changing their direction of locomotion, the positive specimens turning toward the abeye and the negative ones toward the eye-side.

Increase in illumination has no effect on resting specimens regardless as to how rapid or extensive it may be but active specimens respond by changing the direction of motion. This was ascertained with certainty only in negative specimens. These turn toward the abeye-side.

The reaction time is so short that if the hand is moved up and down in front of the microscope as rapidly as possible, alternately increasing and decreasing the luminous intensity, the tail in attached specimens swings from side to side in harmony with the movement of the hand.

If the illumination is gradually changed in either direction there is no response regardless as to how extensive the change may be. The responses to light are dependent upon the time-rate of change in illumination. They are in this respect like the responses in Euglena known as 'shock-reactions' 'schreckbewegung.'

The eye functions as a photo-receptor. If the larvae are attached and a compact source of light is moved in such a way as to produce changes in the illumination of the retina without any change in the illumination of the field responses are induced which are precisely the same as those induced by changing the illumination in the field.

Orientation is brought about by responses (shock-reactions) induced by rapid changes in the illumination of the retina owing to the rotation of the organism on the longitudinal axis resulting in alternate exposure and shading. In negative specimens shading causes the tail to bend toward the eye, exposure toward the abeye-side; in positive specimens precisely the opposite. The one set of responses directs the organism from, the other towards the source of the light. After the tadpoles

are oriented the retina is continuously approximately equally illuminated, the shock reactions cease, and the animals continue on the course established. They remain oriented not because they are held on their course by continuous stimulation, as is so frequently maintained, but because they tend to continue on a direct course when they are not stimulated.

Orientation in this organism is in no way dependent upon a balanced effect of stimuli acting continuously on symmetrically located photoreceptors in accord with the DeCandolle-Verworn theory of orientation accepted by Loeb.

13. Control of morphological polarity by means of the electric current. (Lantern.) E. J. Lund, University of Minnesota.

In 1917 the writer called attention to the similarity between undifferentiated heteropolar individuals of Bursaria, and the ciliary beat in e.g. Paramecium subjected to the electric current. If these phenomena have anything in common then it should be possible to influence the process of establishment of morphological polarity by subjecting differentiating tissues or cells to a direct electric current. This has proven to be the case in internodes cut from Obelia and subjected to a current of proper density. Some of the important facts are:—

A. Regeneration without current. 1. Polyp formation at apical end of an internode occurs before polyp formation at basal end. 2. Apical internodes develop polyps earlier than internodes of basal origin.

B. Regeneration in current. 1. With appropriate current density all polyp formation toward cathode (−) can be delayed if not entirely inhibited, while at the same time polpys may form on anode (+) end in a high percentage of pieces. 2. Under conditions in 1, stolons form and function normally on end toward cathode. 3. Statements 1 and 2 hold for pieces irrespectively of whether basal or apical end is pointed toward anode. 4. A current density barely able to inhibit regeneration in basal internodes can not inhibit regeneration in apical internodes of same branch.

The writer believes that he has succeeded in reversing the original polarity and in determining completely the polarity of regenerating Obelia internodes with an electric current. The establishment of an electrical polarity is probably a fundamental associated condition for development of morphological polarity. The probable relation of the above facts to the earlier discovery of axial differences of electrical potential in uninjured plants and animals by Burdon-Sanderson, Mathews, and others, is apparent.

14. Resistance of fish to different concentrations of salt. R. T. Young, University of North Dakota.

Experiments conducted with several species of fish at the North Dakota Biological Station at Devils Lake indicate that the lake has about reached that degree of concentration (16,000 p. p. m.) which fish can withstand. The sulphates of magnesium and sodium, and chlorides

of sodium, calcium and potassium and sodium carbonate have been used as experimental salts. Osmotic pressure appears to be the main factor in limiting the life of the fish, but a specific toxic effect is also evident.

15. Sterilization by means of spermatoxins. M. F. Guyer, University of Wisconsin.

The action of spermatoxins in vitro has been known since 1899. My problem was to determine how they act in vivo. Spermatoxic serum was prepared by injecting fowls repeatedly with the spermatozoa of rabbits. When, through observation of its effects on spermatozoa in vitro, such serum was found to have become effective it was introduced into the blood stream of four male rabbits at intervals of two or three days until each rabbit had received five or six injections. One rabbit died. One became wholly sterile and the other two partially so. Sterility was determined both by breeding tests and by examination of the semen for spermatozoa. The first rabbit was still sterile, by the breeding test, seven months later when killed for microscopical examination of the testis. The other two still exhibit fertility though it has apparently not been fully restored.

Fowl-serum sensitized against rabbit spermatozoa is also toxic, in vitro, for the spermatozoa of guinea pigs though less so than for those of rabbits.

16. The olfactory reactions of amblystoma tigrinum. J. S. Nicholas (Introduced by Henry Laurens), Osborn Zoological Laboratory, Yale University.

General observations upon the behavior and growth of operated larvae (noseless, eyeless and both noseless and eyeless) show that food or test substances are obtained principally by the sense of sight. Under conditions in which motionless food substances alone are present, the olfactory apparatus functions more effectively in the eyeless than in the normal(seeing) larvae.

Experiments performed with adult animals also show that while the eye is the most important agent in locating food materials yet the nose is capable of detecting and localizing a diffusing food substance.

Tests made with the animals in darkness show a distinct retardation in reaction time when compared with that of the blinded animals. This difference is attributed to dependance upon the sense of sight in the normal animal while in the blinded animal a correlation, by which the olfactory sense is rendered more efficient has been effected.

Experiments performed with diffusing and non-diffusing substances demonstrate the fact that animals possessing olfactory sense (blinded) are stimulated by diffusing substances while those which possess the sense of sight (nose-stopped) are stimulated by non-diffusing substances, particularly if these are in motion.

Air-borne odor streams are effective only when substances possessing irritating properties are used.

The experiments indicate clearly that there is a definite olfactory sense in the larvae and adults of A. tigrinum.

EMBRYOLOGY

17. The shrinkage of embryos in the process preparatory to sectioning. Bradley M. Patten and Rees Philpotte. Western Reserve University School of Medicine.

To ascertain the amount of shrinkage which is induced by preserving embryological material and preparing it for sectioning, series of measurements were made on pig embryos covering each stage of some of the common technique.

The fixatives used were: Zenker, Orth, Tellyesnicky, 10 per cent formalin, formol-alcohol, and Bouin. The crown-rump length of the embryos was measured first in the amniotic fluid, and thereafter following each solution with which they were treated. The average shrinkage was computed in per cent of the original length of the embryo. From these averages graphs were plotted to show the shrinkage encountered at each step of each of the techniques used.

The shrinkage in the bichromate fixatives, Zenker, Orth, and Tellyesnicky, was the greatest encountered, the decrease in crown-rump length ranging from about 30 per cent in 10 mm. embryos to about 20 per cent in 20–25 mm. embryos. The greater shrinkage in the younger embryos is probably attributable to the less compact condition of their mesoderm. Most of the shrinkage in these techniques came in the fixatives themselves.

Fixation in 10 per cent formalin and in formol-alcohol resulted in an initial swelling which was followed by rapid shrinkage during dehydration. The total shrinkage of formalin-fixed material after paraffin infiltration approximated that encountered in material fixed in the bichromate solutions.

The shrinkage in Bouin's fluid was slight but continued during dehydration and infiltration. The total shrinkage in the process was somewhat less than that in the bichromate fixatives.

Comparison of the several graphs shows a tendency for shrinkage in dehydration and paraffin infiltration to be greater following those fixatives which cause less initial shrinkage. This practically nullifies, as far as material for paraffin sectioning is concerned, the supposed advantages of solutions compounded to avoid shrinkage in the fixative.

18. The experimental production of twins in the starfish Patiria miniata: with a discussion of the causes of twinning in general. H. H. Neuman, University of Chicago.

A series of separate twins and of double monsters were produced under three different experimental conditions: *a*) as the result of an extremely belated parthenogenetic development; *b*) as the result of fertilizing Patiria eggs with the sperm of another species of starfish; *c*) as the result of overcrowding normally fertilized eggs. All three methods involve retardation of development, with loss of precise axiate organization at some critical period. Redifferentiation or resumption of axiate organization occurs, but unity of organization has been lost, so that two or more axes or gradients appear instead of the original one. Thus twins or double structures arise.

A series of twin types are produced which represent the results of differences in the earliness of onset of retardation and more or less complete recovery. The series includes completely separated half-sized and quarter-sized blastulae and gastrulae, full-sized gastrulae with two or more archentera, larvae in which the archenteron branches anteriorly into "dicephalous" larvae, and advanced bipennariae with paired, instead of only left-hand, madreporic pores and pore-canals. This physiological theory of twinning agrees with the writer's formerly expressed theory to explain the cause of specific polyembryony in tke armadillo.

19. Bifurcation in the embryos of tubifex. Paul S. Welch, University of Michigan.

Embryonic development in *Tubifex tubifex* (Oligochaeta) has been found to exhibit a striking frequency of abnormalities, chief among which are various forms of body bifurcation. In a series of over 500 cocoons secured from natural habitats and laboratory cultures approximately 20 per cent yielded duplicities. Many types and combinations of type occur, the principal ones being 1) anterior, posterior, or both; 2) simple or compound; 3) equal or unequal; and 4) sagittal, frontal, or both. Anterior doubling appeared about twice as frequently as posterior doubling, and the two types combined in the same individual occurred almost as frequently as the former. Compound bifurcations were not uncommon. Depth of division at the anterior region varied from 1 to 6 somites; in the posterior region from 1 to 17 somites. Bifidity constitutes an almost complete barrier to escape from the cocoon. Careful examination of more than four thousand newly emerged worms yielded only 10 bifid individuals. These anomalies may live within the cocoon long after their escape from the egg membrane, the maximum record being 98 days. Attempts to rear such individuals in laboratory cultures favorable for normal worms of similar age have thus far been unsuccessful and examinations of thousands of both mature and immature worms taken from native habitats and long continued laboratory cultures have failed to reveal a single duplicity. Morphological peculiarities involving the principal internal systems accompany these abnormalities of body form and are given special consideration.

20. Relative sizes of pig embryos. Mary T. Harmon, Kansas State Agricultural College.

The examination of forty-five pregnant pig uteri containing three hundred eleven embryos ranging in length from 18 mm. to 240 mm. and in weight from 0.48 gm. to 872 gm., sixteen litters of pigs born at term consisting of one hundred fifty-nine pigs ranging in weight from 11 oz. to 3 lbs. 10 oz., and three uteri containing embryos of known duration of pregnancy has led to the following conclusions:

1. That there is a great variation in the length and weight of the embryos within a single pig uterus. 2. That this variation is similar

in the early stages of pregnancy as well as the later stages of pregnancy. 3. That this variation is similar in uteri containing few as well as many embryos. 4. That it is also similar in uteri containing the same number of embryos in each horn as those containing more embryos in one horn than in the other. 5. That the variation in length is independent of the variation in weight and vice versa. 6. That the position of the embryo within the horn is not correlated with the variation either in length or weight. 7. That abnormalities are not necessarily correlated with the degree nor direction of the variation either in length or weight. 8. That runts are not more common than unusually large embryos. 9. That the regularity of the distribution of the variation indicates that it may be due to segregation. 10. That the presence of more embryos in one horn of a uterus is a very small factor, if indeed a factor at all, in producing these variations. 11. That the data from the sixteen litters of pigs born at term and those from the three uteri of known duration of pregnancy is in perfect accord with those data obtained from the uteri secured at the packing house. 12. That distribution of the sizes of the pigs born at term strongly indicates segregation in variation in weight. 13. That the age of the mother and the breed of the pigs may affect the limits of size, but not the variation within these limits. 14. That there was one small embryo which may be a case of superfetation and one which may be a case of death in utero and resorption.

21. The effects of transplantation of the several parts of the adult hypophysis into tadpoles in Rana pipiens. BENNET M. ALLEN, University of Kansas.

Microscopic examination of specimens preserved at from two to five months after the operation show that the transplant has lived in normal and apparently functional condition in three-fourths of the specimens studied. This is evidenced not only by their structure but by their vascularization as well.

1. Anterior lobe of the hypophysis transplanted: *a,* Into *normal* tadpoles causes acceleration of development. *b.* Into *pituitaryless* tadpoles induces tendency toward metamorphosis that falls just short of the breaking through of the fore limbs. The thyroid glands show a proportional development, being much larger and containing more colloid than they otherwise show in pituitaryless tadpoles. *c.* Into *thyroidless* tadpoles fails to produce any appreciable tendency toward metamorphosis. Examination of 6 more or less completely metamorphosed specimens in this lot showed in each instance a well developed piece of thyroid gland accidentally left behind in the operation.

These experiments clearly show that the anterior lobe of the hypophysis exerts an influence upon metamorphosis only indirectly through its influence upon the thyroid gland. The anterior lobe stimulates growth especially during early stages; but in these experiments this did not amount to gigantism.

2. Transplantation of the united intermediate and posterior lobes into pituitaryless tadpoles causes them to revert to the original black color after having become white as a result of prior pituitary extirpation. This is due to the action of the intermediate lobe alone as other experiments show. Many such operated tadpoles took on a grey color after several weeks owing to the degeneration of the implanted tissue. Transplantation of the intermediate lobe does not stimulate the development of the thyroid gland; for this reason it likewise has no effect upon metamorphosis.

It seems highly probable that parts of the hypophysis other than the anterior lobe are essential to complete metamorphosis. It is very probable that the pars tuberalis plays a role.

22. Lipolytic effects of egg secretion. O. C. Glaser, Amherst College.

This is a study of hydrolysis in neutral fats and ethyl butyrate under the influence of secretions from arbacia eggs. Definite proof is brought forward showing that hydrolysis is accelerated and that egg secretions contain one or more lipolytic enzymes capable of isolation. An attempt is made to correlate these results with the findings of others and with current theories of fertilization.

23. Proliferation and differentiation in the central nervous system of Amblystoma. G. E. Coghill, Department of Anatomy, University of Kansas.

There is no suggestion of metamerism in either proliferation or differentiation.

In the system taken as a whole there is a fairly constant decrease in proliferation and a fairly constant, but not homogeneous, increase in differentiation throughout the entire developmental period.

Acceleration in rate of differentiation is correlated with retardation in rate of proliferation and vice versa, except for the third interval in the spinal cord.

During the first interval of development there is an increase of differentiation throughout the system which is far greatest in the cerebrum. This is a period of acquisition of new adaptability to environment. During the second interval the greatest acceleration of differentiation is in the rhombencephalon marking an extension and perfection of the responses acquired before. In the third interval the greatest acceleration is in the cerebrum, correlated with the development of locomotion.

Acceleration of differentiation in the cerebrum is correlated with acceleration in the spinal cord and occurs during periods of particular development of longitudinal conduction paths, while retardation of the poles of the system is correlated with marked acceleration in the rhombencephalon during the second interval, when there is particular development of transverse conduction in that region.

There appears to be a wave of acceleration of differentiation, originating in the cerebrum and progressing caudad; a second wave beginning as the first reaches the spinal cord. This suggests that accelera-

tion of differentiation may be rhythmic and a factor in the orientation of neuroblasts and the outgrowth of conduction paths.

The results of these investigations indicate that an exhaustive study of this nature during the earlier periods of development would probably throw new light upon the problem of control in the development of the nervous system.

24. Sex gland transplantation and the modifying effect in rats and guinea pigs. CARL R. MOORE, University of Chicago.

In the white rat, testicular tissue grafted into young, spayed females will persist for a period of nine months. Associated with the testicle graft the behavior of the animal is decidedly male-like.

Ovaries transplanted into young, castrated males will persist and grow for several months. Such an animal, as an adult, exhibits a maternal behavior towards the young. Somatic differences between male and female are too slight to be of value in a differential diagnosis of maleness or femaleness.

In guinea pigs, ovaries grafted into young, castrated males persist for several months and are accompanied by certain somatic modifications in the male; the teats of the mammary glands hypertrophy and compare favorably in size with those of pregnant females, though little or no secretion could be expressed. Psychical modifications of the male are not, in my experience, subject to modification.

Testicular tissue grafted into young, spayed females can be recovered nine months later. No mature sperm were present in the seminiferous tubules but active mitoses were common in cells of the germinal epithelium, a considerable amount of which may remain. Females bearing such testicle grafts exhibit the characteristic male sex behavior (psychical modification) and the external genitalia appear male-like (somatic modification).

In the white rat ovarian grafts will persist for eight months in a male with one normal testicle. Graafian follicles continue their development normally up to the maturation period of the ovum. Subsequently the follicles undergo atresia without ovulation.

There appears to be no deleterious influence of secretions from either sex gland upon the opposite one.

25. The development of connective tissue in the chick embryo. GEORGE A. BAITSELL, Osborn Zoological Laboratory, Yale University.

In a previous communication (Proc. Nat'l. Acad. Sci., VI, 1920) the author has shown that the primitive forerunner of connective tissue in frog embryos is an amorphous, gelatinous, intercellular material which begins to form around the notochord at a very early stage and previous to any cell syncytium. This primitive ground substance is difficult to detect but, with the proper staining, it can be demonstrated in the preserved material and also, as shown in a later communication (Proc. Soc. Exp. Biol. and Med., XVII, 1920), it can be demonstrated in living frog embryos.

These studies have been continued with chick embryos and from the results obtained to date it can be said that an intercellular ground substance is present in the chick embryos and can be readily demonstrated in, for example, a 48-hour stage, in both the living and in the preserved material. This is in agreement with the results obtained by Szily in his work with chick and various other embryos (Anat. Hefte, XXXV, 1907) who said that "Vor dem Auftreten der Mesenchymzellen sind die Lücken und Spalten der Embryonalanlage durch ein feines Fasersystem ausgefüllt." He believed, however, that this ground substance was formed as a result of a fusion of cell processes and was therefore in reality intracellular in its formation.

The evidence from the present studies is that this material is formed in the chick embryo as an intercellular secretion. This is in harmony with the previous results, obtained by the author, with amphibian embryos, as noted above, and is in general agreement with the intercellular theory of connective tissue formation, as stated, for example, by Merkel (Anat. Hefte XXXVIII, 1908).

26. Some habits of cirratulus in relation to fertilization. JOHN W. SCOTT, University of Wyoming.

Previous notes on the development of this annelid were presented in 1911 and 1914. *Cirratulus* lives in the muddy ooze around or near the roots of eel grass and is usually found within two or three inches of the surface. The organic substance in the mud furnishes its food. The breeding season begins early in August and lasts three or four weeks. The cleavage and the behavior of the yolk lobe resemble that of *Dentalium*. At Woods Hole *Cirratulus* is plentiful and easy to collect, but experimental fertilization is difficult due to sensitiveness to external stimuli. Slight mechanical irritation, direct sunlight and, apparently, the presence of the opposite sex, may cause sexual products to be thrown off prematurely. Fully mature eggs are deposited only at night and fertilization occurs at night, or in the morning within a few hours after oviposition. It is possible to secure good material for experimental work early in the morning by handling the worms carefully while collecting, placing each individual in a separate bottle, protecting from sunlight, and keeping each worm isolated over night in a separate finger bowl. Even with these precautions some worms expel immature eggs or sperm. Undoubtedly the worms must come to the surface to discharge their products. So the nocturnal breeding habit is correlated with physiological processes that are active only at night; it is not due directly to a suppression of the reflex by harmful stimuli or we would find ripe eggs in some worms in the day time.

27. Homoplastic and heteroplastic endocrine transplants. W. W. SWINGLE, Osborn Zoological Laboratory, Yale University. (Introduced by R. G. Harrison.)

A. Pituitary glands (anterior lobe) of adult Rana catesbeiana, Rana pipiens, and young Rana climitans were ingrafted intraperitoneally

into immature larvae of the bull frog averaging 55 mm. in length. Two weeks from date of operation hind limbs appeared. The limbs grew and differentiated rapidly and within 30 days from date of operation averaged 12 mm. in length. The tadpoles increased but slightly in size during 40 days of observation. Body emaciation or tail atrophy was not observed. The fore limbs of but one animal appeared. Chief result was effect of the anterior lobe secretion upon the development of limbs. The growth and differentiation of the limbs is much slower than in case of thyroid grafted individuals of same age and size.

B. Pituitary glands (intermediate lobe) of the three species of frogs mentioned were ingrafted into immature larvae. Within 48 hours the experimental animals had changed color from a golden yellow to almost black. This dark pigmentation was retained for eighteen days when most of the larvae resumed normal coloration over night. The larvae showed no increased growth or limb development. Chief result, the effect of the secretion of the intermediate lobe of the pituitary upon the expansion of the Melanophores of the skin.

C. The posterior lobe of the pituitary of three species of frogs ingrafted intraperitoneally into 60 mm. larvae. After 38 days the larvae had not only failed to grow but appeared very much emaciated. No change in pigmentation or limb development occurred. Result: cessation of growth and emaciation.

D. Adrenal glands (cortex and medulla), the paraphysis, and Kiemen Reste were ingrafted with negative results in every case.

E. Thyroid grafts either homo- or heteroplastic are by far the most effective of the endocrine glands in inducing metamorphosis both in regard to the time required and extent to which metamorphic changes occur. Thyroid glands of neotenous larvae are inactive and fail to induce evidences of metamorphosis even when several such glands are ingrafted into the same individual.

It is possible to ingraft a thyroid into an immature larva, induce metamorphosis, re-ingraft the same gland into another larva, induce metamorphosis, then repeat the process again on yet a third animal thus passing the same graft through three individuals.

28. Is the initiatory effect of egg secretion specific? ALVALYN E. WOODWARD, Amherst College. (Introduced by O. C. Glaser.)

Experiments are described in which egg secretions of *Asterias, Arbacia* and *Echinarachnius* have initialed development in eggs of another phylum, namely, on the worm, *Nereis limbata.*

CYTOLOGY

29. Cytology of Anisolabis maritima. (Lantern.) S. I. KORNHAUSER, Denison University.

The chromosomes of the Forficulidae have been described as extremely variable, and the sex chromosome complex as diverse. Thus a detailed study of Anisolabis was undertaken. The diploid chromo-

some number has been determined with certainty as being twenty-five in the male, and twenty-six in the female. The metaphase plates of the first spermatocyte present a puzzling picture, inasmuch as only twelve chromosomal bodies are to be found. On careful analysis it was found that one of the twelve chromosomal bodies was a hexad, the X-chromosome being attached laterally to one-half of an autosomal tetrad. It passes undivided to one of the second spermatocytes. In the interkinetic period it separates so that half the metaphase plates of the second spermatocytes show thirteen chromosomes and half twelve.

In oogenesis each oocyte is accompanied by a single nurse cell. The oocyte and nurse are sister cells, the differentiation being brought about by a cytoplasmic inclusion. Each nurse cell remains attached to its oocyte by means of a root-like outgrowth which extends nearly to the nucleus of the oocyte. During the period of syndesis, the oocyte grows at the expense of the nurse cell, and then as soon as parasyndesis has been effected the nurse cell grows very much more rapidly than the oocyte, the smaller cell of the two preceding in the journey down the ovarial tube. Later on the proportions of the two are again reversed, and finally the nurse cell forms merely a cap anterior to the oocyte.

30. Protoplasmic viscosity changes in the dividing egg of Cumingia. L. V. Heilbrunn, University of Michigan.

Earlier study showed protoplasmic viscosity changes during mitosis. The attempt has now been made to determine the magnitude of these changes and their exact time relation to the mitotic process. Eggs of the lamellibranch Cumingia were centrifuged at short intervals during the time which elapses between fertilization and cleavage. By centrifuging at varying speeds it was possible to determine with some degree of accuracy the comparative viscosity at different stages. At fertilization the egg is in the metaphase of the first polar body mitosis, and the viscosity of its protoplasm is relatively low. Just before the first polar body is given off, the viscosity increases eightfold, it then falls to its original value, increasing eightfold again as the second polar body is given off. This is followed by a slight drop, then a rise in viscosity in the early prophase of the cleavage mitosis. Just before the pronuclei break down, at a stage presumably shortly after the first appearance of the cleavage spindle, the protoplasmic viscosity decreases rapidly until it is as low as in the unfertilized egg. Finally two or three minutes before the cell divides the viscosity suddenly increases until it is nine times that of the unfertilized egg.

31. The structure and division of Trichomonas muris (Hartman). D. H. Wenrich, University of Pennsylvania.

This flagellate protozoon from the coecum of mice is 12–16 microns long by 5–10 microns wide and possesses the following organelles: nucleus; cytosome; and the blepharoplast with its attached structures, the three anterior flagella, the posterior flagellum running as the

margin of the undulating membrane, the chromatic basal rod at the base of the undulating membrane, the axostyle, the outer and inner rows of chromatic granules, and the parabasal body. This last named structure is the true parabasal of Janicki ('11) but appears only after certain methods of technique.

In division, stages comparable to the prophase, metaphase, anaphase and telophase of metazoan cells can be recognized. During prophase, six double (split?) chromosomes are formed, while the caryosome gradually disappears like the metazoan nucleolus. The new chromatic basal rod arises as a row of fine granules with one end of the row attached to the blepharoplast. The new undulating membrane and posterior flagellum develop along with the new chromatic basal rod. A small new blepharoplast is budded off from the parent one and the two remain connected by the paradesmose during division. The nuclear membrane persists throughout mitosis. The behavior of the chromosomes during metaphase and anaphase is similar to that in metazoan cells. The old axostyle degenerates and a new one grows out from each blepharoplast. A new row of chromatic granules is budded off from the inner margin of the chromatic basal rod. Division of the cell body is delayed until two sets of organelles are complete. The nucleus and the cell body are the only parts to divide equationally, all other needed parts being supplied as new outgrowths.

32. A comparison of a Limax Amoebae with tissue culture cells. M. J. Hogue, School of Hygiene and Public Health, Johns Hopkins University.

These amoebae from a 'pure mixed line' average 20μ in their long diameter. They are much smaller than the fibroblasts of an embryonic chick heart which average 50 μ in their long diameter. The fibroblasts attach themselves to the coverslip and spread out very flat, their fine endings being invisible. The amoebae, when grown on agar agar, are flat but they always have a definite outline. The locomotion of the amoebae is visible while that of the fibroblasts is too slow to be noticeable.

When stained with neutral red, both amoebae and fibroblasts have neutral red granules and vacuoles. Neutral red channels have not been seen in the amoebae. Both kinds of cells have mitochondria which stain with Janus black no. 2. In the fibroblast their shape varies greatly from round balls and short rods to long branched forms. In the amoebae only spherical and rod shaped mitochondria are found.

Brilliant crysl blue, methylene blue and neutral red were all used. The stain for the fibroblasts killed the amoebae. After diluting each stain to one-fourth its strength, it colored the amoebae without killing them and gave the same results as the stronger stains did with the fibroblasts. The amoebae is much more sensitive than the tissue culture cells.

33. Unusual tetrads and their bearing on the problem of crossing-over. W. R. B. Robertson, University of Kansas.

Among the ring-like tetrads resulting from the pairing of compound chromosomes, such as occur in Chorthippus curtipennis, there have been found again cases of a condition in which the two strands of one of the members of a pair show one complete revolution about each other which is not present in the strands of the other member of the pair. This torsion occurs, of course, in a region of the tetrad where disjunction has taken place—that is, at an internode between two points of the tetrad which are still in conjunction.

This may mean 1) that the pairing chromosomes were each split and the halves independently twisted about each other before parasynapsis took place, (Robertson, '16, p. 257); or 2) that crossing-over between one strand of each of the conjugants has taken place at some previous time.

If the latter be the correct interpretation, then opposite sides of the ring would each receive one strand of the paternal and one of the maternal pair, and the first division be accordingly equational for the bulk of the tetrad.

The important point, however, is that the crossing-over hypothesis gives a very satisfactory explanation of the abnormality.

34. Spermatogenesis in Cestodes. R. T. Young, University of North Dakota.

Continued investigation of the origin of the sperm cells in cestodes indicate the degenerate character of this process. An abortive maturation mitosis occurs. The spireme breaks up and is distributed through the cytoplasm of the spermatocytes. These fuse to form a cytophore and the sperms appear to arise from the spireme fragments.

35. Conjugation and fission-rate in Arcella vulgaris (Ehrenberg). Hansford M. MacCurdy, Alma College.

In pedigreed cultures of Arcella vulgaris under laboratory conditions the fission rate varies considerably. A general average rate in a non-conjugating line derived from one parent cell was one division for every 2, 56 days. At times when estimated for weekly periods, the fission rate for any one line would increase for a period giving a higher rate and this would be followed by a period of slower divisions. The rate in a parallel line might not be the same.

Conjugation was most often found to occur at times of low fission rate. In many cases this was found to occur at intervals of about a month. There are exceptions. Many exconjugants gave a higher rate of division for a period following conjugation than parallel lines gave for the same period. Some non-conjugants gave a higher rate than some ex-conjugants. Certain nuclear conditions are pointed out and their probable significance considered.

36. The occurrence of precocious and abortive maturation phenomena in female larvae of Rana catesbeiana. W. W. SWINGLE, Osborn Zoological Laboratory, Yale University. (Introduced by R. G. Harrison.)

The writer has shown in earlier work on the germ-cell-cycle of male Rana catesbeiana larvae, the occurrence of a precocious and abortive sexual cycle which culminates in degeneration and resorption of most of the germ cells derived from the primordial entoderm (Keimbahn cells). This larval sexual cycle occurs in immature male tadpoles 45–60 mm. total length, animals which have a year or more of larval life before metamorphosing. Following the degeneration of the spermatocytes, a new generation of germ cells arises, presumably by proliferation of the few lineal descendants of the Keimbahn cells which persist unchanged through the abortive maturation cycle. This new cell generation in many tadpoles just at the time of metamorphosing undergoes a second sexual cycle culminating in the production of normal spermatozoa in the tadpole. The first larval sexual cycle is abortive in almost every feature: great size of the spermatocytes; Urodele type of tetrad, and behavior of the centrosome. The second larval sexual cycle is normal.

Similar phenomena occur in female tadpoles of the same size and age. In undoubtedly female larvae, many of the young oöcytes at the beginning of the growth period pass through all the stages of diakinesis and form first maturation chromosomes of very large size. Most of the cells degenerate at the time of spindle formation owing to the formation of polyasters. In a few cells the maturation process goes as far as the anaphase of the first division, when the spindle apparatus breaks down. The spindles are not eccentric in position, but run from pole to pole of the cell. The tetrad complexes are very large and of the open ring or Urodele type, and bear no resemblance to the rather small dumbbell shaped tetrads characteristic of adult anurans.

It is suggested that the phenomena described here constitute a recapitulation in the germ-cell-cycle of past phylogenetic conditions when the Anura were permanently of the caudate type and reproduced as tad-pole-like forms.

37. Peculiar chromosomal phenomena in a Homopteran. FRANZ SCHRADER, University of Michigan. (Introduced by L. V. Heilbrunn.)

In Pseudococcus nipae, a Homopteran, both sexes have a diploid number of ten chromosomes. In the female, five tetrads are formed and reduction results in the haploid number of five chromosomes as in the ordinary manner. In the male, the growth stages of the spermatocytes show five of the ten chromosomes condensing in advance of the remaining chromosomes. These five chromosomes can be identified in following stages by the tendency to remain in a more or less clumped group. No trace of tetrad formation could be found. The first spermatocyte division is equatorial and ten chromosomes go to each pole. The second division is reductional and this apparently takes place in that the five clumped chromosomes go to one, and the remaining five

to the opposite pole. Early spermatids still show five chromosomes and the formation of spermatozoa seems to follow ordinary lines.

38. The early history of the germ cells in the brook lamprey, Entosphenus wilderi (Gabe), up to and including the period of sex differentiation. PETER OKKELBERG, University of Michigan. (Introduced by Paul S. Welch.)

The germ cells are segregated before the germ layers are definitely established. They are first recognized about the time when the mesoderm separates from the entoderm (embryo about 191 hours old). The definitive germ cells, in both sexes, take their origin from these primordial germ cells and from no other source. Numerous germ cells degenerate in every individual and they never take part in the formation of somatic structures. During the period of sex differentiation two types of cells are found in practically every individual, those which continue to divide and those which stop dividing and enter upon a synaptic and growth phase. The former are taken to be potential male cells (spermatogonia) or indifferent cells and the latter potential female cells (primary oocytes). The relative proportion of the two kinds of cells apparently determines whether the larva shall become a male or a female. Observations seem to warrant the conclusion that each larva carries in it the potentiality of both sexes and that sex, therefore, is not irrevocably fixed at or before fertilization. When a larva becomes definitely established as a male or as a female the opposite sex cells disappear except in the male where rudimentary eggs are frequently found in the adult testis. In the adult condition the number of individuals of each sex is about the same.

GENERAL ZOÖLOGY

39. Something of the life history of a fresh-water medusa. F. PAYNE, Indiana University.

September the twenty-second, 1919, I was notified of the presence of fresh-water medusae in a small artificial lake near Elkhart, Indiana. September the twenty-seventh I visited the lake and found medusae by the thousands. Two weeks later not a medusae could be found. Apparently the cold weather caused their disappearance. All specimens examined were females. A few specimens had been seen the previous summer. The lake, an abandoned clay pit, has been stocked with fish, most of which came from Indiana, but one lot came from a hatchery in Mississippi.

The past summer the lake has been followed closely. The hydroid stage was found and the medusae reappeared. The hydroids have been seen to give rise to sausage shaped buds which break away and develop into other hydroids. Other buds of an entirely different type develop into medusae. The larger medusae have been followed in an attempt to distinguish sexes. Again as in the previous summer only females were present. An examination of posts, limbs, floating

wood, etc., as well as plankton catches at various depths revealed no traces of a larva. So the questions of the males and the development of the eggs into the hydroid still remain unanswered. One is inclined to ask whether the hydroids may not be male and female producing.

In all other finds reported the specimens were all males. The genus is no doubt Craspedacusta. Since seeing the hydroids and medusae I am inclined to think that Craspedacusta and Microhydra are medusa and hydroid stages of one and the same thing.

COMPARATIVE ANATOMY

40. The history of the vertebrate mouth. H. V. Neal, Tufts College.

That the hypophysis of vertebrates represents a paleostoma has been assumed by morphologists on the basis of embryological evidence, supported by that of its functional persistance as an opening in some adult cyclostomes. This hypothesis is still further strengthened by the evidence presented in this paper that the hypophysis is the homologue of the larval mouth (tremostoma) of Amphioxus. The present vertebrate mouth may therefore be considered as the third—or the fourth, if the neuropore be added—mouth in chordate phylogenesis.

41. The origin of the cerebral hemispheres. C. Judson Herrick, University of Chicago.

Assuming that the telencephalon of vertebrate ancestors was the most rostral portion of a neural tube whose walls were uncomplicated by local thickenings or evaginations, it is evident that in different groups of fishes cerebral differentiation has followed several divergent lines. In some ganoids (Acipenser, Amia) and in all teleosts a portion of the lateral wall of the telencephalon adjacent to the lamina terminalis has evaginated to form the olfactory bulb and the remainder of the telencephalon is variously thickened without evagination. The evaginated portions only are the true cerebral hemispheres and the remainder may be termed the telencephalon medium or primitive endbrain. In all vertebrates these two subdivisions of the telencephalon can be recognized, and as we pass from lower to higher forms a progressively larger proportion of the primitive endbrain is joined to the olfactory bulbs in the evaginated cerebral hemispheres.

Some very generalized fishes have, however, extensively evaginated thin-walled cerebral hemispheres instead of massive local thickenings in the primitive endbrain; and this thin-walled form may have been better adapted to oxygenate the brain among the primitive mudfishes which inhabited the drying lakes and streams in the period of increasing aridity of late Silurian geological time and from which the Amphibia arose. After the acquisition of efficient pulmonary respiration in the Amphibia the evaginated cerebral hemispheres, now well supplied with oxygenated blood, differentiated progressively along lines impossible to animals whose forebrain patterns had been stabilized on the teleostean plan.

42. The integumental glands of Alligator mississippiensis. A. M. Reese, West Virginia University.

Three sets of integumental glands are found in the alligator: the dorsal glands, of uncertain function, and two pairs of musk glands.

The dorsal glands are minute spherical structures that are found just under the skin in two rows, one on either side of the mid-dorsal line. They open to the surface through minute pores. They develop as thickenings and invaginations of the lower layer of the epidermis. The wall of the adult gland consists of a sort of loose, stratified epithelium, resembling somewhat the stink gland of the turtle. Since these glands are very small and have, so far as could be determined, no odor, they probably have some other function.

Of the musk glands, the pair that opens into the cloaca is the larger, though the glands that open by a slit on either side of the ventral wall of the throat are of considerable size in large animals. Like the dorsal glands, the musk glands are developed by an invagination of the lower layer of the epidermis, and their walls are composed of somewhat similar though more compact layers of cells. The secretion, or musk, is a smooth, oily substance with the powerful odor characteristic of that well-known extract, and is doubtless used by the sexes, both of which produce it, in locating and following each other during the breeding season, when the glands are said to be most active.

43. The order, time and rate of ossification of the vertebrate skeleton. R. M. Strong, Loyola University School of Medicine.

This is a preliminary report of work in progress on material representing the Amphibia, Reptilia, birds and mammals. In this paper only the work with birds and mammals is considered.

Considerable similarity has been found in the order of ossification, but there are definite differences and some individual variability. In general, the long bones of the limbs show the first ossification. Centers appear about the same time in the clavicle, maxilla, and mandible.

The time of beginning ossification varies greatly in different groups and even in orders. In the birds, the variation is only a few days though the incubation period varies greatly. The first ossification centers appear in the chick about the eighth day of incubation, but not until about the seventeenth day in the rat embryo.

Certain bones vary greatly in the time of ossification. Thus the sternum of the chick embryo begins to ossify about the sixteenth day, but only traces of ossification appear in the duck at about two months after hatching or three months from the first incubation. The white rat sternum has ossification centers on the twentieth day, only about three days after the first centers appear in the rat skeleton.

The rate of ossification varies greatly. It is somewhat more rapid in the chick than in the duck and much more rapid in the rat.

Some observations have been made from the standpoint of the metabolic gradient theory of Child. Ossification is successively later in the vertebrate posteriorly. On the other hand, the appendicular skeleton

precedes the axial slightly, and the distal phalanges ossify before the next row proximally.

44. Primary neuromeres and vertebrate head segmentation. H. W. STUNKARD, New York University. (Introduced by J. S. Kingsley.)

The examination of large numbers of embryos has shown that in Amblystoma and the chick at least, the structures described by Locy and Hill as primary segments can not be regarded as metameric. A repetition of their work, using as far as possible identical means of examination, has in the present case not only failed to verify their findings, but discloses a quite different condition. The three morphological features upon which neuromerism can be based, marginal beadings, external and internal grooves, and cell arrangement, all fail to give evidence to confirm the primary neuromerism of Locy and Hill.

Griggs also endeavored to establish neuromerism as a basis for determining the segmentation of the vertebrate head, describing neuromeres in the procephalic part of the open neural plate of Amblystoma embryos. The median divisions observed in the neural plate of Amblystoma embryos are largely if not entirely due to segmentation of the mesoderm, and can be regarded only as features of secondary importance.

The primary neuromeres of Locy and Hill as well as those of Griggs and other students of neuromerism are irregular in size, inconstant in number, asymmetrical in position, and can not be accepted as trustworthy criteria of the metamerism of the vertebrate head.

45. Studies on lymph nodes; I. Structure, as shown by deposited ink granules. THESLE T. JOB, Loyola University School of Medicine. (Introduced by R. M. Strong.)

Subcutaneous injections of India ink are allowed to be taken up by the lymphatic vessels for varying lengths of time. The course of the ink granules is then traced.

By this method it is possible to show that the posterior or distal part of the node becomes thoroughly congested with ink granules before the anterior or proximal part. In many instances the anterior part never becomes tinged with the ink. The peripheral sinus is not a continuous space over the whole node. The afferent lymph vessels are few in number and enter the periphery at or near a median, longitudinal plane in the posterior part of the node.

Microscopical sections show the distal part of the node more sinusoidal than the proximal part. The proximal part contains more lymph follicles.

46. The topography of the cloaca of the male Necturus in relation to the cloacal glands. ALDEN B. DAWSON, Loyola University School of Medicine. (Introduced by R. M. Strong.)

The external opening of the cloaca of the male Necturus is a longitudinal slit bordered by two inconspicuous lips which, at their caudal

ends, give rise to a pair of soft, non-pigmented, papilla-like projections. The lips are modified further by numerous transverse fissures. Immediately dorsal to the cloacal opening is the cloacal chamber or vestibule which is continued cranially as the cloacal tube. The floor of the cloacal tube has the form of a deep trough with the mucosa thrown up into thin parallel ridges which become interrupted caudally and merge into the tall, slender papillae present on the sides of the cloacal chamber. The roof is modified also by the presence of a deep median groove and, on either side of the cloacal tube, between the dorsal depression and the ventral trough, are two longitudinal furrows.

The cloacal cavity is completely surrounded with masses of long tortuous tubular glands. The large median ventral mass is known as the cloacal gland. Its tubules open on the summits of the parallel ridges and on the tips of the slender internal papillae. The masses of the paired abdominal glands lie ventro-laterally to the cloacal chamber, and their tubules open on the medial surfaces of the paired external papillae. Dorsally there is present a median gland mass, the pelvic gland. This gland shows at least four differentiations, distinguished histologically by the character of the epithelium lining its tubules. There is a small median cranial mass, a very large, median, caudal mass and two lateral masses. All the tubules of the pelvic gland mass open into the roof of the cloacal tube.

PARASITOLOGY

47. Measurements of Trypanosoma diemyctyli from different hosts and their relation to specific identification, heredity and environment. R. W. Hegner, Department of Medical Zoölogy, School of Hygiene and Public Health, Johns Hopkins University.

This investigation involved 1) the problem of the existence of heritably diverse strains of trypanosomes living in the blood of different hosts of the same species, and 2) that of the effects of different environments as represented by the blood stream of different hosts of the same species. A high percentage of infection with Trypanosoma diemyctyli was found in newts (Diemyctylus viridescens) captured in Pennsylvania. Measurements of total length, of portions of the body, and of width showed that specimens from one host animal differed in these dimensions from those in other host animals. For example, specimens taken at random from newt 19 averaged 68.8 microns in total length whereas those from newt 15 averaged only 45.2 microns in total length. Those from newt 19 were also greater in width measuring on the average 3.5 microns in diameter whereas those from newt 15 were only 2.6 microns thick. The different types of trypanosomes obtained from the different newts may belong to different species, or may be races of one species that are heritably diverse in size, or may be sexual phases of a single species, or may differ because of changes due to the environment. Experiments in this laboratory are now in progress to test these various possibilities.

48. Notes on the development and distribution of Oncicola canis (Kaupp 1909). H. J. Van Cleave, University of Illinois.

Acanthocephala have been reported from dogs in various parts of the world. Most of these instances seem to be accidental infestations by species common in other hosts. *Oncicola canis* (Kaupp 1909) is the only species recorded from the dog in North America. Adults of this species have never been found in any other host. Circumstances surrounding the case first described (Parker 1909) indicate the possible association of this parasite with symptoms superficially resembling rabies. Development of this parasite and source of infestation of the definitive host therefore become matters of considerable importance.

The writer has examined larvae taken from the mesenteries and abdominal peritoneum of the armadillo by Albert Hassall. The identity of these larvae and adults from the original lot of specimen of *O. canis* has been determined beyond doubt. The armadillo probably serves this parasite as intermediate host, not primary host as advocated by Travassos for the South American species of the same genus.

Dependence upon the armadillo in its life cycle explains the sharp restriction in natural distribution of this parasite. The undetermined *Echinorhynchus* listed by Ward (1897) from a dog at Lincoln, Nebraska, has been examined and found to be *O. canis*. This occurrence outside the limits of distribution of the larval host is probably due to some sort of accidental introduction.

Parker suggests that many reports of "mad" coyotes may be due to heavy infestations by this species. Unfortunately data of the occurrence in this host are lacking.

49. Dermatobia hominis. Thomas Bryd Magath, Section on Clinical Laboratories, Mayo Clinic, Rochester, Minn.

This paper deals with a general survey of all the reports found in American literature of the parasite, *Dermatobia hominis*. The morphology, life history and distribution of the parasite is discussed. The author's case is reported in detail and the morphology of the larva found is compared with descriptions made by previous authors.

50. The development of the Japanese blood-fluke, Schistosoma japonicum Katsurada in its final host. W. W. Cort, School of Hygiene and Public Health, Johns Hopkins University.

A study of the development of *Schistosoma japonicum* was made from material from experimentally infected mice. After penetrating the skin of the final host the cercariae lose their larval characters. To attain the largest adult size for the species the cercaria must increase in length over one hundred times. Sexual maturity is reached in about twenty days at which time the parasite has increased in size about thirty times. At stages between 0.3 mm. and 0.4 mm. in the livers of the mice, the males show distinctly larger suckers and a broader body than the females and the females have a broader area in front of the union of the

intestinal ceca. As development proceeds the female becomes narrower than the male and round in cross section, while in the male the post-acetabular region gradually becomes flattened and the sides grow up to form the gynaecophoric canal. Larvae as small as 0.3 mm. were found in the livers of the mice up to eighteen days after infection. Since there is little if any growth in size before the larvae reach the liver, and after reaching that organ a very rapid growth ensues, this finding can only be explained by concluding that there is considerable variation in the time taken for the migration from the skin to the liver. The changes in the various organs during the course of development were traced step by step. Differentiation of the reproductive organs comes very late in development.

51. The course of migration of Ascaris larvae from the intestine to the lungs. B. H. Ransom and Eloise B. Cram, U. S. Bureau of Animal Industry.

Stewart ('17) suggested that the most probable path of migration of Ascaris larvae to the lungs after hatching in the small intestine is as follows: Mesenteric veins, portal vein, liver, hepatic vein, vena cava, heart, and pulmonary artery. Yoshida ('19) however, has reached the conclusion that migration of the larvae by way of the circulation if it occurs at all is merely accidental, and states that the newly hatched larvae penetrate through the wall of the intestine into the abdominal cavity, some of them burrowing into various organs such as the liver, spleen and kidney, while others pierce the diaphragm, enter the pleural cavity and finally the lungs. Yoshida also was led to infer that some of the larvae that enter the abdominal viscera, later return to the abdominal cavity and proceed through the diaphragm to the pleural cavity. Ransom and Foster ('20) have recorded two instances in which Ascaris larvae were found apparently in the portal and pulmonary blood vessels. In some recent experiments in which guinea pigs have been killed and examined during the first few days after feeding with eggs of *Ascaris suum* the present writers have repeatedly recovered Ascaris larvae from the portal vein and vena cava but have rarely found them in the abdominal or pleural cavities. Accordingly, the larvae appear to be not uncommonly carried in the circulation, and it would seem that further evidence is necessary before Yoshida's conclusions as to the direct migrations of the larvae through the tissues can be accepted as final.

52. Life history of a new Limax Amoeba. M. J. Hogue, School of Hygiene and Public Health, Johns Hopkins University.

The amoebae were found in the alimentary tract of the oyster. They were grown on various media made up with agar agar and also in liquid media. "Pure mixed lines" were isolated and studied with vital stains and in fixed preparations. The amoeba averages 20.5μ in its long diameter, though it varies considerably, according to the age of the culture. The nucleus is spherical—averaging 2.5μ. It has a large karyosome which is mostly spherical, but often of irregular shape and

size. There is a clear ectoplasm and an endoplasm which contains large refractile granules. No contractile vacuole is present. The amoebae are actively motile, moving usually by a large anterior ectoplasmic pseudopodium.

Mitosis is promitotic. Endogenous and exogenous buds are formed. Binary fission is common. Many multinucleate amoebae are found and an abundance of multiple division.

Cysts are occasionally formed but they are not of common occurrence. The cyst is spherical with a smooth thin wall. It is always uninucleate. Many attempts were made to produce flagellate forms. All were unsuccessful.

53. Correlation of the life-cycle of a parasite with the metamorphosis of its host. NADINE NOWLIN, University of Kansas, Lawrence.

The gregarine, *Lankestrella sciarai* n.sp., inhabits the digestive system of the small fly, Sciara coprophila. With a few exceptions it follows the typical Acephaline life-cycle, beginning as an intracellular parasite and ending as a free Sporozoite. The point of interest here, therefore, is not in the cycle but in the relation of this development to the three stages of its host: the larva, the pupa and the adult.

In the larva are to be found only the stages from entering sporozoite to adult trophozoite, largely an intracellular career. In the pupa, only pseudo-copulation and early cyst formation occur; and in the adult fly, the true copulation of the gametes and the formation of spores. The limitations of development in the parasite are as definite as are the stages of the host.

In other words, the parasite feeds and grows at the feeding and growing stage of the host; prepares for sexual life at a corresponding stage of the host and completes the sexual program in the fully fledged fly, which is itself sexually mature at this time.

This indicates, I believe, a close physiological control of host over parasite, a condition I have suspected from Endamoeba studies but have been unable to demonstrate until now.

54. Observations on the occurrence and life history of Cephalobium microbivorum (Cobb). JAMES E. ACKERT, Kansas State Agricultural College.

Common black field crickets in the vicinity of Manhattan, Kans., are infested with small roundworms which Dr. N. A. Cobb has identified as *Cephalobium microbivorum*, n. g., n. sp. Eighty-five per cent of the crickets, *Gryllus assimilis*, examined between September 19 and October 31 contained these parasites, the average per cricket being 25.7, and the range in all crickets 2 to 91. Examinations of *G. assimilis* at Woods Hole, Mass., Douglas Lake, Mich., and at Rockford, Ill. failed to reveal a single specimen of *C. microbivorum*, while at Falls Church, Va., Doctor Cobb took several specimens from the common black crickets.

The spacious, thin-walled ileum is the habitat of these parasites which are white and thread-like, measuring from 2 to 3 mm. in

length. Their transparent body walls reveal the internal organs, the uteri containing as many as sixty-six thin-shelled eggs in early cleavage stages. Cricket feces contain eggs in the morula stage. In culture media development proceeds through hatching in a few days, and embryos grow slowly on the addition of 0.8 per cent peptone in Dist. H_2O. Both young and adult specimens of *C. microbivorum* occur in the crickets. These parasites may endure the cold weather in the bodies of nymphal crickets (infested in November), some of which survive the winter.

55. Studies on the sheet stomach worm, Haemonchus contortus. JOHN E. GUBERLET, Oklahoma Agricultural Experiment Station.

The stomach worm, *Haemonchus contortus*, is one of the worst enemies of the sheep raiser. Several hundred sheep were treated for the removal of the worms. Copper sulphate, copper sulphate and tobacco, and intra-muscular injections of cacodylate of sodium were used in treating the sheep. Copper sulphate in a 1 per cent solution at the rate of 50 cc. for lambs under one year, and 100 cc. for sheep over one year was found to be 75 to 95 per cent effective. A solution containing 1 per cent copper sulphate and 1 per cent tobacco infusion was found to have an efficiency of 90 to 100 per cent. Cacodylate of sodium was injected intra-muscularly at the rate of 7 grains for an adult sheep. Two or three injections were made at intervals of two or three days with only negative results.

Haemonchus contortus has a periodic seasonal distribution due either to climatic conditions or to the nature of the food at different seasons of the year. Undoubtedly, the nature of the food is a great factor in the gradual removal of some of the worms from the host.

An estimate can be made of the number of stomach worms in the host from the number of eggs in the droppings. Under normal conditions the number of eggs in a gram of fresh droppings corresponds fairly well with the number of adult female worms in the host. There seems to be from 1.5 to 2 times as many females as males. Hence, the number of eggs in a gram of fresh droppings, plus one half to one times that number, gives a fair estimate of the number of adult worms in the host.

56. Acanthocephala from the American eel. H. J. VAN CLEAVE, University of Illinois.

A migratory animal offers many problems with reference to the origin of its parasitic fauna. The parasites of marine and fresh-water habitats usually differ in considerable degree. Among the Acanthocephala few species are common to the two faunas. Because of its wandering between the ocean and freshwater the eel offers exceptional opportunity for a study of the origin of infestation by acanthocephalan parasites.

Various species have been reported from *Anguilla chrysypa*, but many of these have been based upon incorrect identifications. Most of these faulty determinations are due to incomplete descriptions of the older European species which contain little more than generic characters.

Four species of Acanthocephala are known to occur in *A. chrysypa*. A new species, *Tanaorhamphus ambiguus*, was encountered among specimens in the National Museum previously identified as "*E. globulosus*." *Neoechinorhynchus cylindratus* (Van C.) has been reported frequently as the Mediterranean "*E. agilis*." *Echinorhynchus thecatus* Linton and *E. coregoni* Linkins are here reported from the eel for the first time. Of these four species, three occur only in freshwater fishes of North America while the new species represents a genus of which but one other species is known and that parasitizes a single species of freshwater fish.

It is evident that at present the eel does not serve as an agent in mingling marine and freshwater species of Acanthocephala.

EVOLUTION AND GENETICS

57. The production of mosaic males from fertilized eggs in Hymenoptera. P. W. WHITING, St. Stephen's College, Annandale-on-Hudson, N. Y.

An orange-eyed mutation in the wasp, Hadrobracon, acts as a complete recessive to the normal black. Inheritance is "sex-linkoid," the males being haploid and usually parthenogenetically produced. Heterozygous females, isolated as virgin, produce black and orange males in equal numbers. When orange males are mated to black females all offspring are black. In reciprocal mating, daughters are black and most of the sons are orange. A few of the sons, however, are black showing that they come from eggs into which the black-bearing spermatozoon has penetrated. Such anomalous blacks have in some cases bred like black, showing that gonads as well as eyes are paternal in origin. Others have bred like orange, showing that, while eyes are paternal, gonads are maternal. Orange-eyed brothers of anomalous blacks have bred like normal orange, except that in one case such a male bred like a black. Any one male when bred to orange female produces either black or orange daughters, never both; showing that gonad is haploid and either paternal or maternal in origin.

58. The direction and frequency of mutation in a series of multiple allelomorphs. CHARLES ZELENY, University of Illinois.

Full eye, bar eye and ultra-bar eye in Drosophila melanogaster constitute a series of multiple allelomorphs with decreasing facet number and increasing dominance. Bar arose from full and ultra-bar from bar. Observations were made of the direction and frequency of mutation within pure stocks of the members of the series. In the full eye stocks no mutations to bar or ultra-bar were observed during a period of six years among 46,290 counted individuals and among a much larger number of uncounted ones. On the other hand, the reverse mutation from bar to full occurred 52 times among 84,159 individuals or once in 1618, and from ultra-bar to full 5 times out of 8,681, or once in 1736. Correspondingly there were only three mutations of bar to ultra-bar, including the original mutant, among 84,159 individuals or one in 28,053 while the reverse mutation of ultra-bar to bar occurred three times in

8,681 or once in 2,894 and was observed also at another time when the number of individuals examined was not being recorded. Selection for high or low facet number had no effect upon the frequency of any of the mutations.

In this allelomorphic series therefore 1) reverse mutations are much more frequent than the original ones, 2) original progress to ultra-bar is through bar but reversion may go back directly to full as well as through bar and 3) the frequency of mutation is independent of upward or downward selection.

59. On the relation of stale sperm to sterility and sex in ring-doves. Oscar Riddle and Ellinor H. Behre, Station for Experimental Evolution.

The very abnormal sex ratios obtained from hybrid birds by several investigators require the study of all factors possibly concerned. Practical work in pigeon hybridization also sometimes requires a knowledge of the length of time the sperm may remain alive in the female oviduct. On the latter point we find the spermatozoa of the ring-doves (mostly fully fertile hybrids of closely related species) used by us retained their fertilizing power during very nearly eight days, reckoned from the time of isolation of the male to the hour the egg is laid.

"Staleness" of the spermatozoa did not appreciably affect the sex ratio in 213 individual tests made with a dozen pairs of birds. The degree of staleness was known in each test. Some of the sex ratios obtained during the experiment cannot be considered normal but these abnormal ratios have been shown to be associated with other factors investigated earlier. The abnormal sex ratios that have been obtained in previously reported investigations on these doves, and any results that may be later obtained from them or from similar birds, are here shown to be not complicated by effects due to staleness of the spermatozoa.

60. Genetic analysis of low crossover stock produced by selection. J. A. Detlefsen, College of Agriculture, University of Illinois.

Following selection for low crossover values in red-eyed long winged females (Drosophila melanogaster) heterozygous in white miniature, a stock was produced which has given crossover values of about 5–6 per cent for these two genes. The normal value used in plotting chromosome maps is 33 per cent. Matings of red long females from low crossover stock to white miniature males of normal stock gave F_1 females which show an intermediate value. When the F_1 sibs were mated inter se, the total F_2 results also showed an intermediate crossover value. However there was a distinct increase in the range of values.

Matings of red long males from low crossover stock to eosin miniature females of normal stock gave similar results.

61. A note on inheritance of polydactylism in cattle. E. Roberts, College of Agriculture, University of Illinois.

A normal bull mated to a polydactylous cow produced a polydactylous female. This daughter produced from matings to a normal bull, three calves all of which showed the polydactylous condition.

62. Selection in Cladocera (Lantern). ARTHUR M. BANTA, Station for Experimental Evolution.

Some years ago the writer undertook experiments in selection in parthenogenetic pure lines of Cladocera on the basis of a purely physiological character, reactiveness to light. Sixteen lines were subjected to selection for various periods extending over from 18 to 196 generations. In seven of these lines there did not appear any difference in reactiveness between the two strains of the same line. In two lines slight divergences in reactiveness were in the reverse of selection. These divergences, while not large, were fairly consistent. In one of these cases the divergence decreased as the experiment progressed. In five lines there was a possible effect of selection but the evidence is not considered conclusive. In two lines an effect of selection is rather clearly indicated. In one of these the divergence was not large and this case may be disregarded. But in the other the effect of selection is very large and is clearly substantiated. The divergence in this line appeared slowly and increased gradually until during the last months of the experiment the reaction time of the low strain was less than a third that of the high strain. The difference in reactiveness to light was permanent or at any rate persisted for 32 months (112 generations) after selection was discontinued. Return selection was not attempted.

A second series of selection experiments based on an entirely different character is now under way. The character used is the degree of intergradedness of sex intergrade strains of Daphnia longispina. Derived from a common progenitor and reproducing solely by parthenogenesis this would seem a most excellent material for a study of selection. Three strains were selected as high strains and three as low strains, the high and low strains being taken alternately from the six available sister strains. Selection was effective in each case, the individuals of the high strains becoming as high (i.e. as male) as they could be maintained with fair reproductive ability; and the low strains approaching very nearly the condition in which sex intergradedness is not apparent, i.e., most of the individuals showed no sex intergrade characters and the few intergrades were slightly affected. While the facts are as stated, environmental or other factors are influential to such an extent that the curves for the different strains fluctuate somewhat. Further, selection is not effective with equal promptness in every strain though in all cases it has ultimately been effective.

Return selection is also effective. Through selection low strains have been derived from the selected high strains, and high strains from the selected low strains. Two strains have in turn been selected low strains, selected high strains, and selected low strains again.

Thus selection and return selection are equally effective with the amount or degree of sex intergradedness in Daphnia longispina.

63. A study of the character and mode of origin of eighteen mutations in the X-chromosome of Drosophila. H. J. Muller, University of Texas and E. Altenburg, Rice Institute.

Since the eighteen mutants found in the experiment of the writers on mutation frequency were nonselected or random samples of (detectable) mutants in the sex-chromosome, a study of them furnishes quantitative data bearing on the nature of mutations. 1) All were lethals or sublethals. Of the five sublethals, four produced morphological abnormalities. 2) All were completely recessive except one mutant of the yellow mouse type. 3) Half of the loci involved are crowded into the 1.5 units space to the left of white eye (the rest being scattered rather evenly). This indicates that this region of the chromosome is really much longer than the map represents. 4) All the lethals gave negative tests for 'deficiency;' hence deficiencies are evidently much rarer than ordinary lethal mutations. 5) Three lethals were allelomorphs of known non-lethal factors, and two of these lethals were allelomorphs of each other. Of the latter one became dominant in its lethal effect when crossed to a non-lethal allelomorph. 6) Lethals very near 'duplicated' loci remained unaffected by the 'duplication.' 7) Mutation occurs with not markedly different frequency in the two sexes, for seven of the lethals were found in the maternal, eleven in the paternal chromosome. 8) These mutations occur not only near maturation, but also in earlier germ cells, in either sex, as shown by the original appearance of some of the lethals in two sisters simultaneously. 9) Two of the original mutant individuals contained two different lethals at once; in one case these were in opposite chromosomes, in the other case in the same chromosome.

64. Further studies on inheritance of color in the turkey. W. R. B. Robertson, University of Kansas.

The pattern of the black variety is allelomorphic to the pattern of the bronze. Black is almost, not entirely, dominant, there being usually about half-a-dozen bronze feathers widely distributed. F_1 black (bronze) ♂ back-crossed to his bronze dam gave 50 per cent of F_2 bronze and 50 per cent black. An F_1 black (bronze) ♀ by a bronze ♂ gave the same result. The bronze of F_2, mated inter se, gave only bronze, the F_1 blacks gave black and bronze.

Black is likewise allelomorphic to the bourbon red and the narragansett patterns. A bourbon red ♀ by an F_1 black (bronze) ♂ gave 50 per cent bronze-red intermediate, like F_1 of the bronze-by-red cross and 50 per cent a rusty black. An F_1 black (bronze) ♀ by a bourbon red ♂ gave 50 per cent bronze-red intermediate and 50 per cent rusty black. The latter shows a slight trace of barring with white in the primaries. A narragansett ♀ by an F_1 black (bronze) ♂ gave 50 per cent black and 50 per cent bronze.

The last cross shows also that the narragansett pattern is probably allelomorphic to bronze. Narragansett is also likely allelomorphic to bourbon red. Reciprocal crosses gave F_1 much like the narragansett

but with subterminal black bands less intense and slaty regions slightly auburn.

These four patterns evidently form a system of quadruple allelomorphs.

White is recessive to color. A white ♀ by a bourbon red ♂ gave F_1 all bronze-red intermediate. She evidently carried bronze but lacked the factor for color. White ♂ by bronze ♀ gave bronze. F_2 was 75 per cent bronze and 25 per cent white.

65. Experiments with typhoid agglutinins in rabbits. M. F. GUYER and E. A. SMITH, University of Wisconsin.

Experiments are being conducted to determine if immunization against germs of disease, practiced generation after generation, will eventually result in a truly hereditary immunity. Rabbits may readily be sensitized with typhoid vaccine followed by the living bacteria so that their blood-serum diluted 320 to 640 times will agglutinate living typhoid bacilli. Females so sensitized may transmit to their young and even to their grand descendants the ability to agglutinate typhoid bacilli in serum diluted from 60 to 160 times.

After two or three months of development the young of sensitized mothers are likely to show what appears to be a spontaneous rise of titre. If, for example, they have been averaging a titre of 80 for some time, it may rise to 120 or even 160. After a few weeks it drops back again. Rise of titre may be produced by the injection of milk into the blood-stream.

Young from a sensitized mother, when nursed by a normal mother retain a fairly high titre for several months and may even show the spontaneous rise of titre mentioned. Young of a normal mother, when nursed by a sensitized mother acquire a fairly high titre, presumably from the milk of the foster-mother, but lose it rapidly after weaning time.

66. Flat-fish with unusual pigmented areas. (Lantern). ARTHUR M. BANTA, Station for Experimental Evolution.

During the past season many flat-fish have been taken at Cold Spring Harbor which possess more or less pigment on the functionally under (the left) side. In these individuals the pigment region varies from a mere line in the middle region of the caudal fin to complete pigmentation of the under surface except for the head and a small portion of the adjacent body surface. A majority of the unusually pigmented smaller individuals have pigment over a third or more of the under side.

The abnormally pigmented individuals are almost all rather small and are presumably of this season's spring hatch. From an examination of several lots of smaller individuals, altogether about a hundred, approximately one-fourth were found to possess more or less of the unusual pigment distribution. Among about thirty larger individuals examined, presumably of a previous season's hatch, two were

seen with small amounts of pigment caudally. In one of these the pigment was confined to an antero-posterior line in the middle of the caudal fin. In the other the fin and a very little of the caudal body region was pigmented.

All individuals possess normal pigment on the upper side. The pigmented regions on the under side are indistinguishable in appearance from the upper side except at the limits of the pigmented areas. The abnormally pigmented regions are usually rather abruptly defined though there is a slight gradation in density of pigmentation at the margin of the unpigmented areas. The flat-fish possess a black and a reddish brown pigment. At the margin of the pigmented region on the under side the two pigments are not always equally extensive and small patches occur in which only one of the pigments is represented. Wherever either or both pigments occur the amount of pigment present is apparently normal except for a slight grading out at the very edge of the pigmented area.

Six to eight years ago, and to some extent more recently, the writer saw many of those fish but failed to see any with any pigment on the under surface. Fishermen state that they have seen flat-fish "black on both sides" before the present season but agree that they were much less abundant than now. They claim to have seen individuals this season "black all over both sides" but the writer has not seen such.

Flat-fish with more or less pigment on the functionally under side have several times been mentioned in the literature. The points of interest in the present instance, aside from the variation itself, are the occasional segregation of the two pigments near the limits of the unusual pigmented regions, and the abundance of the variation at Cold Spring Harbor during the present year whereas it was at most quite unusual a few years ago.

67. A new type of sex-linked lethal in drosophila. David H. Thompson, University of Illinois. (Introduced by Charles Zeleny.)

A new sex-linked recessive factor has appeared which kills females in double dose and can be recognized in the males which it does not kill. In these males it affects the mesothorax since the wings are held erect and the second pair of legs is feeble. Crosses between heterozygous females and erect males give a sex ratio of one female to two males while other lethals give ratios of two females to one male. Linkage experiments indicate a locus of about 38 in the sex chromosomes. The lethal effect and the character "erect" are manifestations of the same factor as indicated by the absence of crossing over between the two. This erect lethal kills females homozygous for it while other sex-linked lethals kill males. The possibility is offered of producing balanced lethals in the sex chromosomes. Recently a dominant accessory factor has appeared which makes erect dominant in the heterozygous females.

68. The course of evolution in the king snakes. FRANK N. BLANCHARD, University of Michigan. (Introduced by Paul S. Welch.)

The twenty-nine recognized species and subspecies of king snakes, distributed from Canada to Ecuador, present a wide range of variation in structure and color pattern. They afford, therefore, good material for the tracing out of genetic relationships on the basis of structure and distribution.

The conclusions arrived at are based on a careful study of constancy and variation in scutellation, color pattern, and other features, of nearly all the specimens of the genus in American Museums. It is evident that the genus is naturally divided into three main groups or sections, one of which is again divided into three more—that the genus therefore includes five groups of directly related forms. The frequently quoted rule that the occurrence of two different forms of the same genus in the same locality is prima facie evidence that they belong to two different lines of descent, and, conversely, that directly related forms are not found in the same locality, is found to hold for this genus, with one exception for which an interpretation is attempted.

It is further particularly noticable in the more diversified groups, the *triangulum* and *getulus* groups, that the forms are related to each other geographically exactly as one not knowing their distribution would expect them, from a study of structure and pattern, to be related if evolution had proceeded from some one form through successive steps to another distinctively different form. Their present distribution, cannot, therefore, be easily accounted for as due to recent speciation in a formerly widespread homogeneous form, but must rather be attributed to orthogenetic differentiation accompanying migration. A study of variation, distribution, and relationship in the king snakes demonstrates conclusively to the writer the direction of evolution and consequently of migration in each of the five groups of directly related forms. These lines of descent all converge to the southwestern portion of the North American continent and this region is therefore regarded as the center of distribution of the genus. The complete paper will appear under the title "A Revision of the King Snakes: Genus Lampropeltis" as bulletin 114 of the United States National Museum.

ECOLOGY AND ZOOGEOGRAPHY

69. The circulation of water in the Bay of Fundy and its bearing on the distribution of the fauna. JAMES W. MAVOR, Union College.

Investigations into the movements of the water in the Bay of Fundy and its approaches, carried on by Dr. J. W. Mavor for the Biological Board of Canada during the last five years, and more especially during the summer of 1919 and still in progress, have shown a general movement of the water which promises to throw considerable light on the distribution and life-histories of the animal life in the Bay.

Three different and independent methods have been applied to the problem: the actual measurements at different points made with cur-

rent-meters by Dr. Bell Dawson have been treated mathematically so as to eliminate the semi-diurnal oscillations of the tidal stream; a large number of drift bottles have been set out; and lastly, a series of hydrographic sections have been made in the Bay, and from the temperatures and salinities at the different stations the velocities at right angles to the sections have been calculated by the hydrodynamic method developed by Bjerknes.

It seems clear from the calculations from Dr. Dawson's tables, from the drift of bottles and from the hydrographic sections, that the water in the Bay of Fundy has a circulation which may be described as follows: water enters the Bay on its eastern side and flows north-east along the coast of Nova Scotia, it crosses the Bay to the New Brunswick side and flows south-westward out of the Bay, the bulk of the water probably passing to the east of Grand Manan. The rate at which the water flows is probably somewhere between 5 and 10 nautical miles per day, so that the complete circuit probably takes from twenty to forty days.

The drift of the bottles, which left the Bay of Fundy set out at various times during the summer, indicated a surface movement of the water from the Bay of Fundy through the northwestern part of the Gulf of Maine and striking Cape Cod, the rate of this drift being about four nautical miles per day.

The movement of the water described explains the distribution of Chaetognaths and young herring as worked out by Dr. A. G. Huntsman for the Bay of Fundy.

70. Geographical distribution of the Anura and their Opalinid parasites. M. M. Metcalf.

The Amphibia give peculiarly valuable evidence as to land migration routes, for they cannot endure salt or even brackish water. The Opalinids are parasitic exclusively in Anura, excepting three species, two of which are known from Urodeles and one, strangely, from a marine fish. The author has studied 130 species and 20 subspecies of Opalinids from all temperate and tropical portions of the world, southern Asia being the only region outside the northern and southern cold belts from which his material is not quite abundant.

Study of this material and comparison with the known data as to the geographical distribution of the Anura indicates:—

1) That the *Raninae* are a northern and eastern hemisphere subfamily, which have not reached Australasia (except for a single species at the northern tip of Australia), and have not reached South America (except for three species which have entered the northern Andes). 2) That the Bufos arose in the northern hemisphere and were not present in Antarctica, Patagonia and Australia at the time when these were united into an Antarctic continent. The Bufos are now in Patagonia. 3) That the Leptodactylidae arose in Patagonia, at a time when Patagonia was not connected with tropical America, and spread *via* Antarctica to Australia. 4) That later than this, Patagonia having

separated from Antarctica and having united with tropical America, the Bufos passed southward into Patagonia and the Leptodactylids passed northward into tropical America. When the Bufos and Leptodactylids met, the Bufos adopted the Leptodactylid Opalinids, while the Leptodactylids did not adopt the multinucleated Opalinids of the Bufos. 5) That the Hylidae arose comparatively late in the Antarctic continent and did not reach tropical America until Patagonia united with tropical America. That having reached the tropical forests of America the Hylids found here conditions favorable for diversification and rapid increase in numbers. 6) That the genus *Protoopalina* is the oldest of the four Opalinid genera and is found in all parts of the world where climatic conditions are favorable, and has in the past been present in both Arctic and Antarctic regions whose climate now precludes the residence of Anura. 7) That the genus *Zelleriella* arose late in Patagonia and has spread a little into Australia. It spread into tropical America after its union with Patagonia. 8) That the genus *Cepedea* probably arose in the eastern hemisphere and migrated to America, possibly by way of Siberia and Alaska, but more probably both by this route and by a direct land-bridge between Africa and eastern Brazil. Cepedea did not come to America from Africa *via* Antarctica. 9) That the genus *Opalina* is possibly a polyphyletic genus and may have arisen in both the eastern and western hemispheres. 10) That some eastern *Opalinae* have migrated to America. 11) That one American *Opalina* has migrated by way of Alaska and Siberia to Asia and across Europe to France. 12) That the multinucleated Opalinids (*Cepedea* and *Opalina*) entered South America from the north or east and are still scarce in southern South America.

71. Distribution of fishes in Wisconsin lakes. A. S. Pearse, University of Wisconsin.

The distribution of the fishes in six different types of lakes has been studied. Of the larger fishes, the yellow perch is the most abundant species in four of the lakes (Wingra, Mendota, Green, Michigan). The cisco leads in Green Lake, which is clear, deep, and does not stagnate in summer. In Lake Pepin, which is an expansion of the Mississippi River, the most abundant species are the sand-sturgeon and sauger. Lake Michigan and Lake Pepin, which represent primitive lake habitats for their drainage systems, contain many species that are not found in inland lakes. But many of the species which must have come since glacial times from these great reservoirs attain their greatest abundance in inland lakes. The inland lakes all contain more fishes per unit of area than Lake Michigan or Lake Pepin. The lake (Pepin) in which conditions are most like those in a great river has the fewest fishes per unit of area, but contains the largest number of species. Temperature, depth, stagnation, type of bottom, and the abundance of aquatic vegetation are important factors affecting the distribution of fishes.

72. Data on the distribution of fresh-water sponges in North America. FRANK SMITH, University of Illinois.

The most important work on North American fresh-water sponges has been that of Potts and his co-workers in 1880–1890. The most serious defects in their work were due to the failure to recognize *Ephydatia fluviatilis* and *E. mülleri* as distinct species. The former has gemmule spicules with shafts longer than the diameter of the rotules, and the skeleton spicules uniformly smooth; while *E. mülleri* has the shafts usually shorter than the diameter of the rotules, and the skeleton spicules varying from distinctly spined, sparsely microspined, to nearly or quite smooth. Distinctly spined and smooth spicules are often mingled in the same specimen, and in different ratios in different specimens. In the type the skeleton spicules were described as spined. The varietal name *japonica* has been given to specimens with smooth skeleton spicules, found in Japan, and in 1910 the name *Ephydatia japonica* was applied by Annandale to specimens collected in the Potomac River. Material from different parts of Michigan, Illinois, and high mountain lakes of Colorado indicates: that specimens with smooth, or nearly smooth, skeleton spicules are most abundant, but that the ones with spined spicules are similarly distributed; and also that the same body of water may contain both of these kinds of specimens, and still others with spined and smooth spicules mingled in the same skeleton fascicles. *E. fluviatilis* has a similar distribution, but is less abundant than *E. mülleri*.

73. Quantitative and chemical studies of the plankton of Lake Mendota. (Lantern.) C. JUDAY, Wisconsin Geological and Natural History Survey.

These studies were made between April, 1915 and June, 1917. The larger forms, designated as net plankton, were obtained with a regular plankton net, while a centrifuge was used to recover the minute forms, called the nannoplankton. Numerical studies as well as gravimetric determinations were made on both kinds of plankton.

The quantity of dry organic matter in the net plankton varied from a minimum of 73 mgm. per cubic meter of water to a maximum of 1,135 mgm. The average amount for the period covered in these observations was 343 mgm. per cubic meter. Maxima were found in June and November in 1915 and in June and December in 1916. The minima came in April and July–August in 1915 and in March and August, 1916.

The dry organic matter of the nannoplankton varied from a minimum of 795 mgm. per cubic meter of water to a maximum of 3,151 mgm.; the average quantity for the period was 1,630 mgm. The maximum quantity was obtained in April, 1915, and 1916 and in May, 1917. Minima were found in August and early September. The quantity of nannoplankton was always larger than that of the net plankton, but observations on other lakes show that it may be smaller at times.

The net plankton and nannoplankton combined, or the total plankton, gave maxima in April and in October in 1915, with a minimum in August. In 1916 the maxima came in April and late September, with a minimum in late August and early September. During the first half of 1917, there was a minimum in March and a maximum in May.

For the area enclosed by the 20 meter contour line, the dry organic matter of the total plankton ranged from a minimum of about 258 kgm. per hectare (230 pounds per acre) in February to a maximum of 521 kgm. (465 pounds) in December. For the entire lake the former figure is reduced to 141 kgm. per hectare (126 pounds per acre) and the latter to 287 kgm. (256 pounds).

Nearly 53 per cent of the dry organic matter of the net plankton consisted of crude protein and more than 12 per cent of ether extract. The nannoplankton was poorer in nitrogen and ether extract, yielding a little less than 43 per cent of crude protein and 6.5 per cent of ether extract. In the total plankton the crude protein amounted to 44.5 per cent of the dry organic matter and the ether extract to 7.5 per cent.

74. Hydrogen ion concentration of natural waters and the importance of its measurement. (Lantern.) V. E. SHELFORD, University of Illinois and Illinois Natural History Survey.

A comparison of the midsummer hydrogen ion concentrations of various rivers of the United States and Canada which ranges from pH 6.4 to 8.6. Differences form a basis for easy selection of different streams and different tributaries by migrating fish. The relations of hydrogen ion concentration and "alkalinity" to survival and growth of animals in the presence of little oxygen and the sharp reaction of many animals will be urged as reasons for its determination.

75. An analysis of the spawning habits of Chaetopleura apiculata. (Say.) B. H. GRAVE, Wabash College.

1. *Chaetopleura* eggs are probably discharged in nature at the approach of full moon during the months of July and August and to some extent in June and September. The periodicity is not sharply marked although experiments in 1919 seemed to indicate a very distinct periodicity beginning two or three days before full moon and continuing for a week thereafter. No eggs were secured after that date until the near approach of the next full moon. Experimental results in 1920 are less clearly defined but warrant the above conclusion.

2. The sexual products are rarely shed in the day time. They usually begin about eight or nine p.m. and continue for several hours and, at the height of the season, throughout most of the night.

3. The spawning reflex is not induced by a chemical stimulus, as is the case in *Nereis*. Each sex will shed its products when isolated.

4. Laboratory experiments do not furnish reliable criteria for determining normal breeding habits. This is because animals are sensitive to unavoidable shock. It may be shown that oviposition may be induced in many animals by rough and unusual handling, examples of which are given in the text.

5. Periodicity in the shedding of the sexual products seems to be a much more common phenomenon than has formerly been suspected, and further study is likely to reveal this to be a fact. In cases so far studied, the periodicity is related in some way to the cycles of the moon and tides, or to effects upon the development of the sex cells produced by changes in the environment, due to these tidal changes. No adequate explanation of the phenomenon has been proposed but a number of suggestions have been made. Cf. Heppleman, Mayer, Scott, Lillie, Grave. The effects of light, temperature, and pressure have been proposed as effective stimuli. The conditions which surround these species are so varied that the same factors are not equally effective in all cases. It is likely, therefore, that no one explanation is applicable in all instances although the cause of periodicity must be fundamentally the same in all of them. Some kind of shock appears to be the immediate cause.

76. South America and its intercontinental land connections as indicated by a study of the geographic distribution of the Anura and their Opalinid parasites. M. M. METCALF.

A comparison of the geographical distribution of the species of Anura with that of the Anuran Opalinids gives indication of the following relations:—

1) Patagonia was once connected with Australia, doubtless by way of Antarctica, and at that time climatic conditions in Antarctica were favorable for Anuran life. 2) At this time, when Patagonia and Australia were united, Patagonia was not connected with tropical America. 3) When Patagonia lost its connection with Australia it established connection with tropical America. 4) At a period later than the establishment of connection between Patagonia and tropical America, the West Indian land was still united to tropical America. 5) At a period at least as late as this there was land connection between Siberia and Alaska and a mild climate suitable for Anura. 6) For at least two geologic periods there has been, as now, a bar to the migration of moist-skinned Anura (Ranids and Leptodactylids) across northern Mexico and south-western United States. 7) There are five lines of evidence from the Opalinids, but not conclusive, of a connection between Africa and tropical America (not including Patagonia). This land-bridge was not by way of Antarctica. If it existed it was earlier than Pliocene times, probably disappearing as early as early miocene.

77. Relation of temperature and tides to the quantity and distribution of oyster larvae. (Lantern.) E. P. CHURCHILL, University of South Dakota. *Read by permission of the United States Commissioner of Fisheries.*

During the Summer of 1919, studies on this subject were carried on for the United States Bureau of Fisheries, in Great South Bay, Long Island, by the author and Mr. J. S. Gutsell. Stations for observation were located in representative points in the Bay. Collections of the free-swimming oyster larvae were made at these stations every other

day, by pumping known quantities of water through a plankton net. The examination of the larvae was facilitated by straining the material through copper wire sieves of various sizes of mesh. The number, size and stage of development of the larvae in each sample were recorded together with data, relative to the temperature, tide, weather, etc., at the station where the collection had been made. About two hundred and fifty samples of water were taken during the oyster spawning season, which extended that year from about June 5th to July 17th. The spawning began about the time the water temperature reached 70 degrees Fahrenheit and proceeded briskly while it ranged from seventy to seventy-five but slowed down or ceased when the temperature fell to seventy-five or above, July 2nd to 6th, the bulk of the spawn was thrown out in the course of two or three days. The length of the free swimming period of the larvae was twelve to fourteen days.

The currents caused by the tide carried the larvae about in the Bay. The tidal movements were such as to cause the larvae to become concentrated in certain definite areas of the Bay. In general the larvae were most numerous at a point about half way out along the buoyed channel from West Sayville to Ocean Beach and diminished in numbers from this point each way to the ends of the channel. Beds of oyster shells, which had been placed in various parts of the Bay, were examined after July 17th for the presence of attached larvae or set. It was found that set was most abundant on the beds in the region where the larvae had been most numerous and that beds outside of the channel, where few larvae had been found, received a smaller amount of set and those at the ends of the Bay, two or three miles from the channel, received practically none at all.

78. Some reactions of the jelly-fish, Aequorea. A. O. Weese and M. T. Townsend.

Aequorea tends to select sea water with a pH of about 8.1, i.e., about the normal hydrogen ion concentration of Puget Sound sea water. When the animal encounters water with a pH lower than 6.8, it reacts by turning its oral surface up and actively moving downward and finally ceasing to pulsate. In water of constantly increasing pH pulsation ceases at a pH of about 8.9, but the avoiding reaction does not usually follow.

If the temperature is gradually raised the animals seek lower levels at about 20°C. A temperature as low as −2°C. did not prove fatal.

In water of gradually decreasing salinity Aequorea seeks a lower level at a point corresponding to 20 gms. of NaCl per liter or two-thirds the concentration of normal sea water.

79. Longevity of Trogoderma tarsale *larvae without food.* J. E. Wodsedalek, University of Idaho.

About three years ago the writer published the results of the first series of starvation experiments with Trogoderma tarsale larvae. In that case the last of a number of specimens lived, without a particle to eat, for a period of five years and two months. The larvae concerned

had undergone considerable disturbance occasioned by long trips on a train and variations in temperature which reduced their longevity.

In this second and more extensive series of experiments the larvae were undisturbed and lived at a fairly constant room temperature. Eight classes of specimens ranging from 1 to 8 mm. in length, each comprising ten individuals, were selected for starvation until death occurred. The larvae gradually decreased in size and practically all of them dwindled down to the hatching size of 1 mm. before dying. Two out of ten of the largest specimens lived over six years without food, the last one dying after six years, two months, and seven days of starvation.

In another experiment groups of specimens varying in size from 2 to 8 mm. in length are undergoing periods of "feasting and fasting." The larvae in various stages of starvation when given plenty of food again begin to grow in size. For example, some of the large specimens are on their way to their fourth 'childhood' after having attained the maximum larval size three times; while specimens originally 4 mm. in length are on their way to their ninth 'childhood' after reaching 4 mm. eight times. The status of various other phases of the problem will be briefly presented.

80. The problem of the local distribution of Thias lapillus imbricatus *(Lamarck.)* HAROLD S. COLTON, University of Pennsylvania.

The delicate imbricated variety of *Thais* (*Purpura*) lapillus forms a large element of the Thais population of the littoral formation of certain sheltered bays and harbors of the Coast of Maine and is nearly absent from other bays and harbors offering a similar environment. Although commonly absent from the rocks exposed to surf it has been found in proportions ranging from 17 per cent to 95 per cent on the exposed rocks of Duck Island and Grand Manan. This fact upsets the commonly accepted explanation of the distribution of Thais imbricatus, viz., that the delicate shells are destroyed by wave action on the exposed coast and natural selection is the important factor determining their presence or absence. Again, in two adjoining bays the correlation between the exposure to wave action and the proportion of imbricated forms is very different:—in one bay the correlation is almost perfect and in the adjoining bay nearly zero.

Whatever the factor is, which determines the distribution of the imbricated variety, an examination of 20,000 shells from 106 different stations proves that it is not wave action alone.

SYMPOSIUM OF FERTILIZATION

81. A critical discussion of the chromidial formation of nuclei, of autogamy, and of the multiple division of polyenergid nuclei, with especial reference to the unity of the mechanism of heredity in the Protozoa and Metazoa. CHARLES ATWOOD KOFOID. Univ. of California, Berkeley.

The fundamental unity of the mechanism of heredity in the Metazoa and Metaphyta as a whole is becoming increasingly evident. This includes the mitotic process, maturation, and fertilization with attendant synapsis and segregation and diploid and haploid stages of the nuclei of the organism. Attached to this conception of this mechanism as more or less essential corollaries are the current conclusions as to the individuality of chromosomes and their continuity from generation to generation, the particulate theory of inheritance, the localization of genes, and the phenomenon of crossover.

The primacy of the Protista in the evolutionary process leads us to turn to them for the developmental steps in the perfection of the hereditary mechanism. We find the evidence somewhat diversified as might be expected, and not a little chaotic. For example, if the de novo origin of nuclei of vegetative cells of gametes, and even of zygotes, occurs as described in Protozoa, the hereditary mechanism operates in the category of chromidial granules instead of in that of chromosomes and their localized parts. The chromidial hypothesis of Hertwig, as elaborated by Goldschmidt and the Schaudinn-Hartmann school, is based on the conception of a cytoplasmic idiochromatin of nuclear origin. These cytoplasmic inclusions in flagellates and rhizopods, especially in those of the digestive tract of vertebrates, are basophilic chromatoidal substances appearing in the cytoplasm during an acidophile phase of the nucleus. They are often associated, as they are in Arcella, with the coincident appearance of glycogen in the cytoplasm. I find no evidence that they ever as such form secondary nuclei, or that the primary nucleus disappears during their formation. The so-called secondary nuclei of supposedly chromidial origin are in reality derived as usual by mitosis. The evidence thus far presented of the de novo chromidial origin of protozoan nuclei is wholly inadequate to establish this hypothesis.

The occurrence of autogamy with attendant automixis in the Protozoa lacks thus far any conclusive evidence of synapsis or segregation and in no case is there a demonstration of the expected haploid—diploid sequence based on the accurate determination of the numbers of the chromosomes in the supposed periods of maturation and syngamy. In the autogamy of Actinosphaerium, as described by Hertwig, there is a significant resemblance to endomixis in Paramoecium and inadequate evidence of syngamy of the supposed pronuclei. The autogamy in Endamoeba coli of Schaudinn confuses the life-cycle of that species with phases of the yeast-like Blastocystis of human faeces. That described by Prowazek for Bodo is based on an analogous confusion, and that described by him for Trichomastix confuses in one cycle a flagellate and an amoeba. On the other hand, the autogamous cycle of the rhizomastigote flagellate described by Goldschmidt appears to involve a case of parasitism. Parasitism by the problematical organism Sphaerita may also easily give rise to appearances of the de novo origin of secondary nuclei in its host.

The multiple autogamy recently described in Endamoeba coli in Japan is a condition often seen in the cysts of this amoeba when recently divided nuclei are crowded within an encapsuling zone of chromatoidal material. It is possibly pathological or degenerative, but is certainly not autogamy.

The coincident multiple mitosis of so-called polyenergid nuclei in the Radiolaria, wherein one large nucleus breaks up into many small ones without mitosis, is under suspicion as a base of parasitism similar to that by Amœbophrya, or by a parasitic dinoflagellate. More conclusive evidence is needed to establish the normality of this phenomenon. We may therefore dismiss the chromidial origin of nuclei, chromidiogamy, autogamy, and multiple nuclear division in the Protozoa as without adequate cytological foundation.

82. The initial event in fertilization. FRANK R. LILLIE.

The initial event in fertilization has a primary significance because all others depend for their occurrence upon it and for their degree of efficiency upon its quantitative value. The initial event also displays a high degree of simplicity in relation to subsequent events.

Two new methods of study are applied in this paper to its study: first, the effect of copper salts upon the fertilization reaction; second, a comparison of the relative degrees of specificity between sperm agglutination by egg secretions and the fertilization reaction itself.

Copper has an incomparably greater effect on the initial reaction in fertilization than on later stages in Arbacia. It may therefore be used for an analysis of this reaction. The results indicate the presence of a copper-avid substance in the cortex of the egg that is responsible for activation.

The specificity of sperm agglutination by egg secretions between two species of Strongylocentratus is found to be of the same order as fertilization specificity.

The copper-avid substance of the cortex of the egg is to be identified with the sperm agglutinating substance of egg secretions and with the fertilizing of previous papers.

83. The susceptibility of the inseminated egg to hypotonic sea-water. A contribution to the analysis of the fertilization-reaction. E. E. JUST.

I

1. The uninseminated egg of Echinarachnius parma may be exposed to the action of hypotonic sea-water without loss of fertilization capacity. The length of this exposure depends upon the degree of dilution of the sea-water. Thus, eggs may be exposed to *full strength* tap-water for one to one and one half minutes and retain capacity for fertilization. On return to normal sea-water such eggs develop despite the abnormal behavior of the germ nuclei or their chromosomes which a study of sectioned material has revealed.

2. Immediately after insemination in sea-water and up to about forty seconds after insemination, eggs of Echinarachnius exposed to

the action of hypotonic sea-water develop on return to normal sea-water. As in the case of the uninseminated egg, the per cent of cleavage and of larvae as well as the normality of development depends upon the length of time the eggs have remained in the hypotonic sea-water and upon the degree of dilution employed.

3. Eggs two minutes after insemination are likewise resistant to the action of hypotonic sea-water as measured by the per cent and quality of development on return to normal sea-water. In these three stages, the eggs, as so far measured, are about equally resistant to a given dilution of sea-water. After two minutes following insemination, however, the egg increases in resistance to hypotonic treatment. Ten to fifteen minutes after insemination, for example, the egg is most resistant.

4. With eggs exposed to hypotonic sea-water forty to one hundred twenty seconds after insemination, the case is quite different. During this period of about eighty seconds the eggs are highly susceptible to the action of dilute sea-water. Thus, eggs exposed to *full strength* tap-water forty seconds after insemination show one hundred per cent disintegrated in fifteen seconds or less; whereas the uninseminated egg does not disintegrate in tap-water under two minutes. The susceptible period is thus sharply defined.

II

1. The period of susceptibility to hypotonic sea-water exhibited by inseminated Echinarachnius eggs runs parallel with the separation of the so-called fertilization membrane through the break-down of the cortex of the egg. If eggs are exposed to hypotonic sea-water at the moment of membrane separation they cytolyze rapidly. With complete membrane separation the period of susceptibility ends; the egg is again resistant.

Eggs exposed to dilute sea-water during the period of membrane separation cytolyze by bursting at that point where the membrane was lifting at the moment of exposure. Thus, if the membrane is off just above the point of sperm entry, where membrane separation begins, the cytoplasm breaks down at this point; if the membrane is one half off, the break-down takes place in the angle between the separated membrane and the egg; and if the membrane is lifting from its lats point of attachment to the egg (the pole opposite to that of sperm entry) the break-down is at this point. All zones of the cortex from which the membrane has lifted are resistant; likewise, are those to which the membrane is still stuck. The susceptibility of the egg is, therefore, clearly due to weakness of the cortex only in that zone from which the membrane is lifting.

2. Can this low resistance to hypotonic sea-water be an osmotic phenomenon? What is the rôle of the so-called fertilization membrane during this period? How does this period of low resistance compare with that just before cleavage? What are the physical properties of the egg substance at this time? May we compare the wave of susceptibility sweeping around the egg with other forms of protoplasmic

transmission? These are but a few of the questions that this study raises and attempts to answer. By far the most important question at this time, however, is this: how does this period of susceptibility relate itself to the fertilization-reaction?

III

1. The increase in susceptibility of the inseminated egg to hypotonic sea-water is not the cause of fertilization; nor is it the fertilization-reaction itself.

A. The egg is fertilized before the membrane lifts. (1) A mass of data shows that inseminated eggs exposed to hypotonic sea-water prior to membrane separation are capable of developing. (But this, however, is also true of the uninseminated egg.) (2) Polyspermy is blocked before membrane separation begins. (3) Other visible cortical changes precede those leading to membrane separation. (4) The case of Platynereis shows us how rapidly sperm may effect profound changes in the egg.

B. That the susceptibility of the inseminated Echinarachnius egg is purely a cortical (local) phenomenon and not the fertilization-reaction is supported by observations of the susceptibility of Arbacia and Nereis eggs to hypotonic sea-water.

2. It is therefore held as hitherto that the fertilization reaction is practically an instantaneous reaction between sperm and an ovogenous substance, fertilizin.

84. Fertilization and egg-secretions. Otto Glaser, Amherst College.

1. Egg secretions have been known for some time from the eggs of at least ten species of echinoderms, two of annelids, two of tunicates, and one of molluscs. To this list I now add another mollusc, the oyster, and two vertebrates, the fish, Fundulus heteroclitus, and the frog, Rana pipiens.

2. The importance of these secretions in the initiation of development has been demonstrated by a variety of methods. One of these, that of washing the eggs, has been criticized because in the eighteen to thirty-six hours required to remove all traces of their exudates, the eggs themselves may undergo serious deteriorations of other sorts. By using running sea water, I have been able to shorten this period to three or four hours, and by removing their jelly and exposing the eggs to charcoal, I have succeeded in sterilizing them completely in thirty minutes.

3. The secretions agglutinate spermatozoa and initiate development. These effects are due, not to one substance with two side-chains, as postulated in the Fertilizin Theory, but to two chemical entities, the lipolysin on the one hand, and the agglutinin, on the other.

4. The lipolysin is a lipolytic ferment and catalyses the hydrolysis of the esters of the lower as well as the higher fatty acids. It may be that more than one ferment is involved.

5. The agglutinin very possibly is also a ferment, but the process which it catalyses has not yet been found.

6. A study of the specificities of fertilization must take account of the lipolysin and of the agglutinin. With respect to the former, it is possible to employ lipolysins derived from the eggs of arbacia, asterias, and of the oyster, for the purpose of increasing the fertility of Echinarachnius eggs partially sterilized by the removal of their own secretions; With respect to the agglutinin, it is possible to increase very greatly the success of crosses between Echinarachnius parma and Arbacia punctulata, if one kind or the other of their sex cells, but especially the spermatozoa, are treated with species-true egg secretion prior to insemination. It appears therefore that the agglutination reaction involves specific features because species-true agglutinin has effects quantitatively and perhaps qualitatively different from those of heterogeneous agglutinins.

Whatever transformations our views on the initiation of development may undergo within the next few years, the zone within which we must seek for understanding is now marked off by the reaction capacities of perfectly definite physiological compounds.

85. The chromosomes in fertilization. C. E. McClung.

1. The process of fertilization consists essentially in the introduction into the egg of a simplex series of chromosomes, duplicating the series left there by oögenesis. Little or no other material is carried by the spermatozoön.

2. Since genetic experiments indicate the equivalence of male and female in heredity, the importance of the chromatin is demonstrated.

3. Behavior of characters in inheritance, indicating factor differences and groupings, are paralleled by conditions of structure and behavior of the chromosomes.

4. The chromosomes introduced by the spermatozoön are reduced to the smallest volume and contain chromatin in the most condensed condition.

5. This chromatin quickly absorbs fluid from the egg cytoplasm and forms a nuclear vesicle in which the chromosomes later appear in the size, form, and number that marked them in the spermatid.

6. Upon union of the egg and sperm pronuclei the paternal chromosomes may remain distinctly grouped and this segregation may be followed through many generations of cells.

7. The individual paternal chromosomes may be traced into the body cells of the embryo and are found later in the germ cells.

8. During the many generations between the ovum and the adult organism in which maturation occurs, the chromosomes have reproduced themselves, each time under different conditions in the organization and constitution of the body, so that in the germ cells they must emerge somewhat different in character from what they were on entering.

9. At the period of maturation in the germ cells, however, the homologous elements from the two parents unite together in the most inti-

mate manner but without the loss of their individual identity, thus completing the process of union inaugurated by fertilization.

10. Still, distinguished by characteristics of form, size, and behavior, they are then segregated by chance and distributed again into mature germ cells in a simplex series. Through these they may be traced again into another generation of organisms where they repeat the series of processes.

11. Fertilization, although not necessary to reproduction, and omitted in parthenogenesis, is required in biparental inheritance and there serves the essential purpose of introducing the necessary duplicate control factors—the chromosomes.

86. Chromatic Material in Hybridization. David H. Tennent. Bryn Mawr College, Bryn Mawr, Pa.

Closeness of relationship is by no means indicative of the readiness with which the initial impulse to development may be received, nor a sure criterion of the extent to which it may proceed. Some species hybridize in nature; some eggs show a cortical block which may be removed readily by various methods. The entrance of a spermatozoan following the removal of the cortical block, may result in development, or it may result in an instantaneous, or in a slower but none the less complete cytolysis of the egg.

In some crosses, in which a specialized type of development is superimposed on a more general type, development proceeds regularly up to the point of deviation of special from general. Internal block may become effective apparently at any stage after the entrance of the spermatozoan. Many degrees of inhibition, ranging from failure of the germ nuclei to unite to failure of synapsis have been described, but no methods of overcoming its effects have been devised.

From our knowledge of straight fertilization and of cross fertilization we have come to look upon development as an attribute of the egg. In eggs of Arbacia fertilized by sperms of Moira, an interordinal cross, a rhythmic appearance of basophilic bodies in the cytoplasm may be seen. A similar phenomenon has been described in many species fertilized eggs. By the application of binuclearity hypotheses, founded in part on the chromidial hypothesis, to the metazoan cell, these basophilic bodies have been explained as somatochromatin or trophochromatin. The evidence for the emission of chromatin as such from the nucleus is not convincing. In the conditions of the experiment mentioned, a foreign enzyme was introduced. Its presence produced a coalescence of granules into coarsely dispersed aggregates. It is suggested that this coalescence is a result of dehydration due to the activity of the foreign enzyme in the cytoplasm. These bodies are regarded as synthesized in the cytoplasm. We cannot hope to distinguish between more than very widely spaced steps in synthesis within the cell by methods of staining. The egg and spermatozoan may form a harmonious system, the degree of harmony being a function of cytoplasmic substrate and nuclear enzyme.

PAPERS RECEIVED TOO LATE TO CLASSIFY

88. A new method of ingestion—exemplified in the ciliate Frontonia. WILLIAM M. GOLDSMITH, Southwestern College. (Introduced by S. O. Mast.)

Observations and experimentations were made upon the Ciliate Frontonia while these organisms were ingesting, not only their favorite food—Euglena, diatoms, desmids, and Oscillatoria, but also silk and cotton fibers and various other foreign materials. The ingestion of blue-green algae, which produced fantastic figures through the contortion of the organism by the variously colored food, furnished the most conclusive demonstrations of the method involved, as the process continued a greater length of time and involved more factors. 1. The cilia of the cyto-pharynx exert a direct pull upon the incoming food. 2. The locomotory cilia drive the organism forward and thus force the food into the mouth. 3. The end of the fiber enters the mouth and passes antero-dorsally, after which the Frontonia swings around through an angle of 180 degrees, using the mouth as a pivot, thus permitting the fiber to pass down the dorsal side and to exert a pressure on the body wall at the extreme posterior end. Such points of contact are known as tension points, as a play of the three factors heretofore considered tends only to push the fiber through the body wall. 4. A series of short contractions of the body wall assists in relieving the tension point. 5. Cyclosis aids by moving the end of the fiber around the wall, thus forming a spiral and making further ingestion possible.

89. Copper: its occurrence and rôle in insects and other animals. RICHARD A. MUTTKOWSKI. (Introduced by J. E. Wodsedalek.)

During investigations on the respiration of insects the writer incinerated both the blood and entire specimens of over 40 species of insects, representing thirteen of the orders. These were selected to represent the greatest variety of stages, habitats, and food habits. The ash was analyzed for copper, on the supposition that the copper serves as the nucleus of a respiratory protein, hemocyanin. The amount found in all cases was nearly equal to that of the control substance, crayfish blood.

In addition to insects and crayfish, plankton Crustacea, spiders, centipeds, and other Arthropods were incinerated for copper, with positive results. To see how widely copper was distributed in the animal world the writer incinerated Volvox, Ascaris, Lumbricus, snails, slugs, mice, human and snake blood, with positive results in each case, except human blood.

For the sources of this copper the water, soil, and plants were tested. All three showed copper in varying amounts.

These results indicate that the element copper has a wider distribution in living organisms than heretofore accepted. Its function has been definitely ascertained for mollusks and a few Arthropods, where it forms the nucleus of a respiratory protein, hemocyanin. Its rôle in

other Arthropods is probably identical. For the amount of copper present in the various Arthropods incinerated was nearly equal to or exceeded that of the control substance, crayfish blood.

The copper ion is probably inactive in plants. It is known to be highly toxic to lower plants. Yet even these (Penicillium) contained copper.

90. The post-embryonic development of the compound eye of Drosophila melanogaster. Joseph Krafka, Jr., University of Georgia. (Introduced by Charles Zeleny.)

A histological study has been made of the development of the compound eye of Drosophila melanogaster Meig. The embryoblasts are present at the time of hatching, although the ommatidia are not completely metamorphosed until the late pupal period. The segmented condition of the optic ganglion, before definitive visual structures appear, suggests that the formation of the latter may be under the control of the nervous system. A marked reduction in the size of the optic ganglion in the bar-eyed mutant shows that the hereditary factor involves more than the facet-number.

91. The sex element in the flash of the firefly. C. R. Fountain, Mercer University.

While engaged in researches as to the nature of the chemical reactions in the firefly which cause the flash of light, I had trouble during the latter part of the evening in catching as many fireflies as I needed in my researches. I hit upon the following method which I thought would be of some interest to the Zoologists, in view of the many discussions about the synchronous nature of the flashes.

Late in the evening the male fireflies are nearly all up in the tops of the trees and have ceased to flash. By means of a small flashlight held between my fingers I imitated the flash of the male firefly. Instantly there would be a number of responses from the grass, sometimes from a distance as great as 100 yards. Walking toward the nearest one I would flash several times, getting a response every time until I was within five feet, when apparently I was recognized as an imposter. However, I was always able from that distance to find the firefly by the faint continuous glow, or by the light of the flashlight itself. By this means I invariably found the female. In the early evening those flashing as they rise are mostly the males, at least 15 times as many males as females can be caught.

I might state that my observations seemed to prove that the chemical process is a slow oxidation, but I have not yet learned the exact type of chemical reaction that gives off this very efficient radiation. I am much more hopeful of finding out this now than when I first began the work.

AMERICAN SOCIETY OF ZOÖLOGISTS

CONSTITUTION, OFFICERS AND LIST OF MEMBERS OF THE SOCIETY

CONSTITUTION

ARTICLE I

NAME AND OBJECT

Section 1. The Society shall be called the "American Society of Zoölogists."

Sec. 2. The object of the Society shall be the association of workers in the field of Zoölogy for the presentation and discussion of new or important facts and problems in that science and for the adoption of such measures as shall tend to the advancement of zoölogical investigation in this country.

ARTICLE II

MEMBERSHIP

Section 1. Members of the Society shall be elected from persons who are active workers in the field of Zoölogy and who have contributed to the advancement of that science.

Sec. 2. Election to membership in the Society shall be upon recommendation of the Executive Committee.

Sec. 3. Each member shall pay to the Treasurer an annual assessment as determined by the Society. This assessment shall be considered due at the annual meeting and the name of any member two years in arrears for annual assessments shall be erased from the list of members of the Society, and no such person shall be restored to membership unless his arrearages shall have been paid or he shall have been re-elected.

Sec. 4. Foreign Zoölogists, not members of this Society, may be elected Honorary Fellows upon unanimous recommendation of the Executive Committee by a majority vote of the members present at any meeting of the Society. Honorary Fellows shall not be required to pay dues.

ARTICLE III

OFFICERS

Section 1. The officers of the Society shall be a President, a Vice-President a Secretary-Treasurer and the members at large of the Executive Committee.

Sec. 2. The Executive Committee shall consist of the President, the Vice President, the Secretary-Treasurer and five members elected from the Society at large. Of these five members, one shall be elected each year to serve five years. If any member at large shall be elected to any other office, a member at large shall be elected at once to serve out the remainder of his term.

Sec. 3. These officers shall be elected by ballot at the annual meeting of the Society and their official terms shall commence with the close of the annual

meeting, except that the Secretary-Treasurer shall be elected triennially and shall serve for three years.

Sec. 4. The officers named in Section 1 shall discharge the duties usually assigned to their respective offices.

Sec. 5. Vacancies in the board of officers, occurring from any cause, may be filled by election by ballot at any meeting of the Society. A vacancy in the Secretary-Treasurership occurring in the interval of the meetings of the Society may be filled by appointment, until the next annual meeting, by the Executive Committee.

Sec. 6. At the annual meeting the President shall name a nominating committee of three members. This committee shall make its nominations to the Secretary not less than one month before the next annual meeting. It shall be the duty of the Secretary to mail the list of nominations to all members of the Society at least two weeks before the annual meeting. Additional nominations for any office may be made in writing to the Secretary by any five members at any time previous to balloting.

Article IV

MEETINGS OF THE SOCIETY

Section 1. Unless previously determined by the Society the time and place of the annual meeting of the Society shall be determined by its Executive Committee. Special meetings may be called and arranged for by the Executive Committee. Notices of such meetings shall be mailed to all members of the Society at least two weeks before the date set for the meeting.

Sec. 2. Sections of the Society may be organized in any locality by not less than ten members, for the purpose of holding meetings for the presentation of scientific papers. Such sections shall have the right to elect their own officers and also associate members; provided, however, that associate membership in any section shall not confer membership in the Society.

Article V

QUORUM

Twenty-five members shall constitute a quorum of the Society and four a quorum of its Executive Committee.

Article VI

CHANGES IN THE CONSTITUTION

Amendments to this Constitution may be adopted at any meeting of the Society by a two-thirds vote of the members present, upon the following conditions:

(a) The proposed amendment must be in writing and signed by at least five members of the Society.

(b) This signed proposal must be in the hands of the Secretary at least one month before the meeting of the Society at which it is to be considered.

(c) The Secretary shall mail copies of the proposed amendment to the members of the Society at least two weeks before the meeting.

BY-LAWS

DUES

(1) The annual dues for members, unless remitted or changed by the vote of the Society, shall be seven dollars.

SECRETARY-TREASURER

(2) The duties and privileges of the Secretary-Treasurer shall be as follows:

(a) He shall keep the records and be in charge of the funds of the Society.

(b) At the annual business meeting he shall present a statement to date of the funds of the Society.

(c) Whenever the proper officers of a number of related societies shall have a conference with a view to determining a common time and place for the several annual metings, he shall act as the delegate or representative of this Society. (See also 5-a.)

(d) He shall employ a typewriter or printer whenever in his judgment such employment will expedite the business of the Society, and

(e) He shall be reimbursed out of the funds of the Society for expenses incurred in attending meetings of the Society.

AUDITING COMMITTEE

(3) The President shall annually appoint an auditing committee of two, who shall audit and report upon the financial record and statement of the Secretary-Treasurer at the meeting for which they were appointed.

(4) The National Research Council allows the Society three representatives on the Division of Biology and Agriculture. Of these three representatives, one shall be elected each year to serve three years. The method of election shall be the same as that used in the election of the officers of the Society.

AFFILIATION WITH THE AMERICAN SOCIETY OF NATURALISTS

(5) It shall be the policy of the Society to hold meetings in both Eastern and Central-Western territory, and the distribution of the meetings between the two territories shall be determined in general on the basis of the representation of Eastern and Western members in the Society. See also 2-c.

PROGRAM RULES

(6) In matters relating to programs for annual meetings the following rules shall be observed:

(a) Papers shall be listed and presented according to subject matter in the following groups: 1. Comparative Anatomy; 2. Embryology; 3. Cytology; 4. Genetics; 5. Comparative and General Physiology; 6. Ecology, and 7. Miscellaneous, or other groups at the discretion of the Secretary-Treasurer.

(b) Whenever conditions require it the Executive Committee shall schedule two or more groups for the same hour and rearrange the program to bring together papers on subjects of more general interest for meetings of the whole Society. The Committee, however, is instructed to avoid conflicts as much as possible.

(c) Papers shall be listed in their respective groups in the order received. When a member offers more than one paper those following the one designated first shall be placed at the end of the list and shall not be read until all first papers by members shall have been twice called for.

(d) All papers not read when called for as listed shall be placed at the end of the group list, and, if not read when called for the second time, they shall be read by title only.

(e) The titles of "introduced" papers shall be listed in the groups after the titles of papers to be read by members. Such papers shall be read by title only in case the entire program cannot be completed during four regular sessions for reading papers.

(f) Fifteen minutes shall be the maximum time allowed for the presentation of a paper.

(g) Abstracts of papers for publication in the proceedings of the Society must be handed to the Secretary-Treasurer or his representative before final adjournment of the annual meeting.

HISTORICAL REVIEW

A review of the historical antecedents of the present American Society of Zoölogists will be found in *The Anatomical Record* for January, 1917. The list of officers and meeting places of the present Society found in the same place is brought up to date and reprinted here.

OFFICERS AND LIST OF MEMBERS[1]

American Society of Zoölogists (Amalgamated)

MEETING PLACES

1914—Philadelphia	1917—Minneapolis	1919—St. Louis
1915—Columbus	1918—Baltimore	1920—Chicago
1916—New York		

Officers for 1920

President	Gilman A. Drew
Vice-President	Caswell Grave
Secretary-Treasurer	W. C. Allee

Executive Committee

	Term expires
R. P. Bigelow	1920
H. V. Wilson	1921
M. M. Metcalf	1922
George Lefevre	1923
C. M. Child	1924

Representatives of the Society in the Division of Biology and Agriculture of the National Research Council

	Term expires
F. R. Lillie	1923
M. F. Guyer	1921
G. H. Parker	1922

EDITORIAL BOARD OF THE JOURNAL OF MORPHOLOGY

Managing Editor (Term expires 1926) . . . . . C. E. McClung

Associate Editors

To serve until 1922	Gary N. Calkins J. S. Kingsley William Patten
To serve until 1923	E. G. Conklin M. F. Guyer W. M. Wheeler
To serve until 1924	C. A. Kofoid F. R. Lillie J. T. Patterson

[1] The data given in this list is based on the last preceding list published in *The Anatomical Record*, Vol. 17, No. 5, with such corrections and additions as have come to the attention of the Secretary. Please notify the Secretary of errors in this copy of the membership list that they may be corrected in the next published list.

HONORARY MEMBER

JAMES VISCOUNT BRYCE, HINDLEAP, FOREST ROW, SUSSEX, ENGLAND

LIFE MEMBERS

ANDREWS, ETHAN ALLEN, Ph.B. (Yale), Ph.D. (Johns Hopkins), Professor of Zoölogy, *Johns Hopkins University, Baltimore, Md.*

DEAN BASHFORD, A.B. (College of City of New York), A.M., Ph.D. (Columbia), Professor of Vertebrate Zoölogy, Columbia University; Curator Emeritus of Fishes and Reptiles, *American Museum Natural History, Riverdale-on-Hudson, New York.*

HENSHAW, SAMUEL, Director of Museum of Comparative Zoölogy, *8 Fayerweather Street, Cambridge, Mass.*

MAYER, ALFRED GOLDSBOROUGH, M.E. (Stevens Inst. Tech.), Sc.D. (Harvard), Director Department Marine Biology, Carnegie Institution of Washington, *276 Nassau Street, Princeton, N. J.*

METCALF, MAYNARD MAYO, A.B., Sc.D. (Oberlin), Ph.D. (Johns Hopkins), Orchard Laboratory, *128 Forest Street, Oberlin, Ohio.*

MOORE, J. PERCY, Ph.D. (Pennsylvania), Professor of Zoölogy, *University of Pennsylvania, Philadelphia, Pa.*

STILES, CHARLES W., A.M., Ph.D. (Leipzig), S.M., S.D. (Wesleyan), Professor of Zoölogy, United States Public Health and Marine Hospital Service, Hygienic Laboratory. *Twenty-fifth and E Streets, N.W., Washington, D. C.* (October 1–May 1); *Wilmington, N. C.* (May–October 1).

MEMBERS

ACKERT, JAMES EDWARD, A.B., A.M., Ph.D. (University of Illinois), Professor of Zoölogy, Parasitologist Agr. Exp. Station; *Kansas State Agricultural College, Manhattan, Kan.*

ALLEE, WARDER CLYDE, S.B. (Earlham College), S.M., Ph.D. (Chicago), Professor of Biology, Lake Forest College, *Lake Forest, Ill.*

ALLEN, BENNET MILLS, Ph.B. (DePauw), Ph.D. (Chicago), Professor of Zoölogy, *University of Kansas, Lawrence, Kan.*

ALLEN, EZRA, A.B., A.M. (Bucknell), Ph.D. (University of Pennsylvania), Professor of Biology, *Ursinus College, Collegeville, Pa.*

AREY, LESLIE BRAINERD, Ph.D. (Harvard), Professor of Microscopic Anatomy, *Northwestern University Medical School, 2431 Dearborn St., Chicago, Ill.*

BAITSELL, GEORGE ALFRED, B.S. (Central College, Iowa), M.A., Ph.D. (Yale), Assistant Professor of Biology, Yale University, *Osborn Zoölogical Laboratory, Yale Station, New Haven, Conn.*

BAKER, ARTHUR CHALDEN, B.S.A. (Toronto University), Ph.D. (George Washington University), Entomological Assistant, *Bureau of Entomology, Washington, D. C.*

BANTA, ARTHUR MANGUN, A.B., A.M. (Indiana), Ph.D. (Harvard), Resident Investigator, *Station for Experimental Evolution, Carnegie Institution, Cold Spring Harbor, Long Island, N. Y.*

Barrows, William Martin, B.S. (Michigan Agricultural College), S.B., S.M. (in biology) (Harvard), Department of Zoölogy and Entomology, *Ohio State University, Columbus, Ohio.*

Bartelmetz, George W., Ph.D. (Chicago), Associate Professor of Anatomy, *University of Chicago, Chicago, Ill.*

Baumgartner, William Jacob, A.B., A.M. (Kansas), Associate Professor of Zoölogy, *University of Kansas, 1209 Ohio Street, Lawrence, Kan.*

Beckwith, Cora Jipson, B.S. (Michigan), M.A., Ph.D. (Columbia), Associate Professor of Zoölogy, *Vassar College, Poughkeepsie, N. Y.*

Bellamy, Albert W., Ph.D. (Chicago), Instructor in Zoölogy, *University of Chicago, Chicago, Ill.*

Bigelow, Maurice Alpheus, B.S. (Ohio Wesleyan), M.S. (Northwestern), Ph.D. (Harvard), Professor of Biology, Teachers College, *525 West 120th Street, New York City.*

Bigelow, Robert Payne, S.B. (Harvard), Ph.D. (Johns Hopkins), Associate Professor of Zoölogy and Parasitology, *Massachusetts Institute of Technology, Cambridge, Mass.*

Binford, Raymond, B.S. (Earlham), S.M. (Chicago), Ph.D. (Johns Hopkins), President of Guilford College, *Guilford, N. C.*

Boeck, William Charles, B.S., M.A., Ph.D., Hygiene Laboratory, U. S. Public Health Service, Washington, D. C.

Boring, Alice Middleton, A.B., A.M., Ph.D. (Bryn Mawr), *Wellesley College, Wellesley, Mass.*

Boyden, E. A., Ph.D. (Harvard), Assistant Professor Comparative Anatomy, *Harvard Medical School, 61 Clark Street, Newton Center, Mass.*

Bruner, Henry Lane, A.B. (Abingdon), Ph.D. (Freiburg), Professor of Biology, *Butler College, 324 South Ritter Avenue, Indianapolis, Indiana.*

Budington, Robert Allyn, B.A., M.A. (Williams), Professor of Zoölogy, *Oberlin College, Oberlin, Ohio.*

Burrows, Montrose T., A.B. (Kansas), M.D. (Johns Hopkins), *Washington, University Medical School, St. Louis, Mo.*

Byrnes, Esther F., Ph.D. (Bryn Mawr), *193 Jefferson Avenue, Brooklyn, N.Y.*

Calkins, Gary N., B.S. (Mass. Inst. Tech.), Ph.D. (Columbia), Professor of Protozoölogy, *Columbia University, New York City.*

Calvert, Philip Powell, Ph.D. (Pennsylvania), Professor of Zoölogy, *University of Pennsylvania, Zoölogical Laboratory, Philadelphia, Pa.*

Carothers, E. Eleanor, A.B., A.M., Ph.D., *Zoölogical Building, University of Pennsylvania, Philadelphia, Pa.*

Carpenter, Frederic Walton, B.S. (New York University), A.M. Ph.D. (Harvard), Professor of Biology, *Trinity College, Hartford, Conn.*

Cary, Lewis R., B.S., M.S (Maine), M.A., Ph.D. (Princeton), Assistant Professor of Biology, *Princeton University, Princeton, N. J.*

Casteel, Dana Brackenridge, A.B. (Allegheny), A.M. (Ohio Wesleyan), Ph.D. (University of Pennsylvania), Associate Professor of Zoölogy, *University of Texas, Austin, Texas.*

Castle, William E., A.B. (Denison), A.M., Ph.D. (Harvard), Professor of Zoölogy in *Harvard University, Payson Road, Belmont, Mass.*

CHAMBERS, ROBERT, JR., M.A. (Queen's University, Can.), Ph.D. (Munich), Assistant Professor in Anatomy, *Cornell University Medical College, 28th Street and First Avenue, New York City.*

CHAPMAN, ROYAL N., B.A., M.A. (Minnesota), Ph.D. (Cornell), Assistant Professor of Animal Biology; Assistant Entomologist, *Experiment Station, University of Minnesota, Division of Entomology, University Farm, St. Paul, Minn.*

CHESTER, WAYLAND MORGAN, A.B., A.M. (Colgate University), Professor of Biology, *Colgate University, Hamilton, N. Y.*

CHILD, CHARLES MANNING, Ph.B., M.S. (Wesleyan), Ph.D. (Leipzig), Professor of Zoölogy, *Hull Zoölogical Laboratory, University of Chicago, Chicago, Ill.*

CHURCHILL, EDWARD PERRY, A.B. (Iowa), Ph.D. (Johns Hopkins), Professor of Zoölogy, *University of South Dakota, Vermillion, S. D.*

CLEMENS, LUCY, B. A. (Smith), M. A. (Mt. Holyoke), Ph.D. (Cornell), *307 Dupont St., Toronto, Ont., Canada.*

COE, WESLEY R., Ph.D. (Yale), Professor of Biology, *Yale University, New Haven, Conn.*

COGHILL, GEORGE E., A.B., Ph.D. (Brown), Professor and Head of Dept. of Anatomy, University of Kansas, *R.F.D. 9, Lawrence, Kan.*

COLE, LEON J., A.B. (Michigan), Ph.D. (Harvard), Professor of Experimental Breeding, *College of Agriculture, University of Wisconsin, Madison, Wis.*

COLTON, HAROLD SELLERS, B.S., M.A., Ph.D. (Pennsylvania), Assistant Professor of Zoölogy, *University of Pennsylvania, Philadelphia, Pa.*

CONGDON, EDGAR DAVIDSON, A.B., A.M. (Syracuse), Ph.D. (Harvard), Leland Stanford Jr. University, *330 Coleridge Ave, Palo Alto, Cal.*

CONKLIN, EDWIN GRANT, Ph.D. (Johns Hopkins), Sc.D. (Pennsylvania), Professor of Biology, *Princeton University, Princeton, N. J.*

COPELAND, MANTON, S.B., S.M., Ph.D. (Harvard), Professor of Biology, *Bowdoin College, Brunswick, Maine.*

CORT, WILLIAM WALTER, A.B. (Colorado College), A.M., Ph.D. (University of Illinois), School of Hygiene and Public Health, *310 W. Monument St., Baltimore, Md.*

CRAMPTON, HENRY EDWARD, A.B., Ph.D.; (Columbia), Professor of Zoölogy, *Barnard College, Columbia University,* Curator of Invertebrate Zoölogy, *American Museum of Natural History, New York City.*

CROZIER, WILLIAM JOHN, B.S. (College of the City of New York), A. M., Ph.D. (Harvard), Professor of Zoölogy, *Rutgers College, New Brunswick, N. J.*

CURTIS, MAYNIE ROSE, A.B., A.M., Ph.D. (Michigan), Assistant Biologist, *Maine Agricultural Experiment Station, Orono, Maine.*

CURTIS, WINTERTON CONWAY, A.B., A.M. (Williams), Ph.D. (Johns Hopkins), Professor of Zoölogy, University of Missouri, *208 Hicks Ave., Columbia, Mo.*

DAHLGREN, ULRIC, A.B., M.S. (Princeton), Professor of Biology, Princeton University, *204 Guyot Hall, Princeton, N. J.*

DANIEL, J(OHN) F(RANKLIN), S.B. (University of Chicago), Ph.D. (Johns Hopkins), Professor of Zoölogy, *University of California, 100 Woodmont Ave., Berkeley, Cal.*

DAVENPORT, CHARLES BENEDICT, Ph.D. (Harvard), Director of Department of Experimental Evolution, Carnegie Institution of Washington, *Cold Spring Harbor, Long Island, N. Y.*

DAVENPORT, GERTRUDE CROTTY, B.S. (University of Kansas), *Cold Spring Harbor, Long Island, N. Y.*

DAVIS, HERBERT SPENCER, Ph.B. (Wesleyan), Ph.D. (Harvard), Professor of Zoölogy, *University of Florida, Gainesville, Fla.*

DAWSON, JAMES ARTHUR, A.B. (Dalhousie), A.M., Ph.D. (Yale), Professor of Biology, Dalhousie University, *Halifax, Nova Scotia, Canada.*

DAY, EDWARD CARROLL, A.B. (Hamilton), A.M., Sc.D. (Harvard), Assistant Professor of Zoölogy, *University of Cincinnati, Cincinnati, Ohio.*

DETLEFSEN, JOHN A., A.B. (Dartmouth), A.M., Sc.D. (Harvard), Assistant Professor of Genetics, University of Illinois, College of Agriculture, *506 Michigan Avenue, Urbana, Illinois.*

DETWILER, SAMUEL RANDALL, Ph.B., M.A., Ph.D. (Yale), Instructor in Anatomy, *Peking Union Medical College, Peking, China.*

DODDS, GIDEON S., B.A., M.A. (Colorado), Ph.D. (Pennsylvania), Assistant Professor of Zoölogy, *University of West Virginia, Morgantown, West Virginia.*

DOLLEY, WILLIAM LEE, JR., A.B., A.M. (Randolph-Macon), Ph.D. (Johns Hopkins), Professor of Biology, *Randolph-Macon College, Ashland, Va.*

DREW, GILMAN A., B.S. (State University of Iowa), Ph.D. (Johns Hopkins), Assistant Director, *Marine Biological Laboratory, Woods Hole, Mass.*

DUNN, LESLIE CLARENCE, S.B. (Dartmouth), S.M., Sc.D. (Harvard), Poultry Biologist, *Connecticut Agricultural Experiment Station, Storrs, Conn.*

DUPORTE, ERNEST MELVILLE, M.Sc., B.S.A. (McGill), Instructor in Zoölogy and Entomology, Macdonald College, *Macdonald College, P. O. Province Quebec, Canada.*

EDMONDSON, CHARLES HOWARD, Ph.B., M.S., Ph.D. (Iowa University), Professor of Zoölogy, Director of Research Laboratory, *College of Hawaii, Honolulu.*

EDWARDS, CHARLES LINCOLN, B.S. (Lombard and Indiana), A.M. (Indiana), Ph.D. (University of Leipzig), Director, Department of Nature Study, Los Angeles City Schools, *1032 West 39th Place, Los Angeles, Calif.*

EIGENMANN, CARL H., A.B., A.M., Ph.D. (Indiana), Research Professor and Dean of the Graduate School, *Indiana University, Bloomington, Ind.*

ENDERS, HOWARD EDWIN, B.S. (Lebanon Valley College), B.S., M.S. (Michigan), Ph.D. (Johns Hopkins), Associate Professor of Zoölogy and in charge of Biology, Purdue University; Summer School Staff, Dept. Zöology, Indiana University, *107 Fowler Ave., West Lafayette, Indiana.*

ERDMANN, RHODA, Ph.D. (Munich), Berlin-Wilmersdorf, Nassauische str. 17II, Germany.

ESTERLY, CALVIN O., A.B., A.M. (California), Ph.D. (Harvard), Zoölogist, Scripps Institution, La Jolla, California; Professor of Biology, *Occidental College, Los Angeles, Calif.*

FASTEN, NATHAN, B.S. (College of City of New York), Ph.D. (Wisconsin), Associate Professor of Zoölogy, *Oregon Agricultural College, Corvallis, Ore.*

FERRIS, HARRY BURR, B.A., M.D. (Yale), E. K. Hunt Professor of Anatomy, Medical Department, Yale University, *395 St. Ronan, New Haven, Conn.*

FOX, HENRY, B.S., M.A., Ph.D. (University of Pennsylvania), Professor of Biology, *Mercer University, Macon, Ga.*

FRASER, CHARLES MCLEAN, A.B., A.M. (Toronto), Ph.D. (Iowa), Professor of Zoölogy, University of British Columbia and Director of Marine Biological Station, Nanaimo, *University of B. C., Vancouver, B. C., or Biological Station, Nanaimo, B. C.*

GAGE, SIMON HENRY, B.S. (Cornell), Emeritus Professor of Histology and Embryology, *Cornell University, Stimson Hall, Ithaca, N. Y.*

GALLOWAY, THOMAS W., A.B., A.M., Ph.D. (Cumberland), A.M. (Harvard), Litt.D. (Missouri Valley), *105 W. 40th St., New York City.*

GARMAN, HARRISON, D.Sc. (Kentucky), Professor of Entomology and Zoölogy, Kentucky Agricultural Experiment Station; State Entomologist, *Lexington, Ky.*

GEE, WILSON, B.S. (Clemson), M.A. (University of South Carolina), Ph.D. (University of California), *University of South Carolina, Columbia, S. C.*

GEROULD, JOHN H., Litt.B. (Dartmouth), A.B., A.M., Ph.D. (Harvard), Professor of Biology, Dartmouth College, *36 Occom Ridge, Hanover, N. H.*

GLASER, OTTO CHARLES, A.B., Ph.D. (Johns Hopkins), Professor of Biology, *Amherst College, Amherst, Mass.*

GOLDFARB, A. J., B.S. (College of City of New York), Ph.D. (Columbia), Professor of Biology, College of City of New York, *Convent Ave., New York City.*

GOLDSCHMIDT, RICHARD B., Ph.D. (Heidelberg), Kaiser Wilhelm Institut für Biologie, *Berlin-Lichterfelde 3, Germany.*

GOLDSMITH, WILLIAM MARION, B.Pd., A.B., A.M., Ph.D. (Indiana), Professor of Biology, Southwestern College, *Winfield, Kansas.*

GOODALE, HUBERT DANA, Ph.D. (Columbia), Research Biologist, *Massachusetts Agricultural Experiment Station, North Amherst, Mass.*

GOODRICH, HUBERT BAKER, B.S. (Amherst), M.A., Ph.D. (Columbia), Instructor in Zoölogy, *Wesleyan University, Middletown, Conn.*

GRAHAM, JOHN YOUNG, Ph.D. (Munich), Professor of Biology, *University of Alabama, University, Ala.*

GRAVE, BENJAMIN H., B.S., (Earlham) M.S. (Carleton), Ph.D. (Johns Hopkins), Professor of Zoölogy, *Wabash College, Crawfordsville, Ind.*

GRAVE, CASWELL, B.S. (Earlham), Ph.D. (Johns Hopkins), Professor of Zoölogy, *Washington University, St. Louis, Mo.*

GREGORY, LOUISE H., A.B. (Vassar), A.M. Ph.D. (Columbia), Instructor in Zoölogy, *Barnard College, New York City.*

GRIER, NORMAN MCDOWELL, S.B., A.M., Ph.D. (Pittsburgh), Professor of Biology, Washington and Jefferson College, *Washington, Pa.*

GRIFFIN, LAWRENCE EDMONDS, A.B., Ph.B. (Hamline), Ph.D. (Johns Hopkins), Reed College, Portland, Oregon.

GROSS, ALFRED O., A.B. (Illinois), Ph.D. (Harvard), Assistant Professor of Zoölogy, *Bowdoin College, Brunswick, Maine.*

GUBERLET, J. E., A.M., Ph.D. (Illinois), Parasitologist, Oklahoma A. and M. College, *Stillwater, Okla.*

GUDGER, E. W., B.S., M.S. (Nashville), Ph.D. (Johns Hopkins), *American Museum of Natural History, New York City.*

GULICK, ADDISON, A.B. (Oberlin), A.M. (Harvard), Ph.D. (Wurzburg, Germany), Assistant Professor in Physiology, University of Missouri, *903 Lake Street, Columbia, Mo.*

GUYER, MICHAEL F., B.S. (Chicago), A.M. (Nebraska), Ph.D. (Chicago), Professor of Zoölogy, *University of Wisconsin, Madison, Wis.*

HALL, MAURICE CROWTHER, S.B. (Colorado), M.A. (Nebraska), Ph.D. (George Washington), D.V.M. (George Washington), Senior Zoölogist, Zoölogical Division, *Bureau of Animal Industry, Washington, D. C.*

HAMAKER, JOHN IRVIN, A.B. (Kansas), A.B., A.M., Ph.D. (Harvard), Professor of Biology, Randolph-Macon Woman's College, *12 Princeton Street, Lynchburg, Va.*

HANCE, R. T., A.B., M.A., Ph.D. (Pennsylvania), Instructor in Zoölogy, University of Pennsylvania, *Zoölogical Laboratory, University of Pennsylvania, Philadelphia, Pa.*

HANSON, FRANK BLAIR, A.B. (George Washington), A.M. (Illinois), Ph.D. (American University), Assistant Professor of Zoölogy, Washington University, *Department of Zoölogy, Washington University, St. Louis, Mo.*

HARGITT, CHARLES W., Ph.D. (Ohio University), Professor of Zoölogy, Director of Laboratories, *Syracuse University, Syracuse, N. Y.*

HARGITT, GEORGE THOMAS, Ph.B. (Syracuse), A.M. (Nebraska), Ph.D. (Harvard), Associate Professor of Zoölogy, *Syracuse University, 909 Walnut Ave., Syracuse, N. Y.*

HARMON, MARY THERESA, A.B., M.A., Ph.D. (Indiana), Assistant Professor of Zoölogy, *Kansas State Agricultural College, Manhattan, Kan.*

HARPER, EUGENE HOWARD, A.B. (Oberlin), A.M. (Harvard), Ph.D. (Chicago), *Bedford, Va.*

HARRISON, ROSS GRANVILLE, Ph.D. (Johns Hopkins), M.D. (Bonn), Bronson Professor of Comparative Anatomy, Yale University, *142 Huntington St., New Haven, Conn.*

HARTMAN, CARL G., Ph.D. (Texas), Associate Professor of Zoölogy, University of Texas, *511 W. 33rd St., Austin, Texas.*

HEATH, HAROLD, A.B. (Ohio Wesleyan), Ph.D. (Pennsylvania), Professor of Invertebrate Zoölogy, Leland Stanford University, *Palo Alto, Calif.*

HECHT, SELIG, Ph.D. (Harvard), Assistant Professor of Physiology, *Creighton Medical College, Omaha, Neb.*

HEGNER, ROBERT W., B.S., M.S. (Chicago), Ph.D. (Wisconsin), Instructor in Protozoölogy, School of Hygiene and Public Health, *Johns Hopkins University, Baltimore, Md.*

HEILBRUN, L.V., Ph.D. (Chicago), Instructor in Zoölogy, *University of Michigan, Ann Arbor, Mich.*

HENCHMAN, ANNIE P., *Box 34, Jaffrey, N. H.*

HERRICK, CHARLES JUDSON, Ph.D. (Columbia), Professor of Neurology, Anatomical Laboratory, *University of Chicago, Chicago, Ill.*

HERRICK, FRANCIS HOBART, A.B. (Dartmouth), Ph.D. (Johns Hopkins), D.Sc. (Pittsburgh), Professor of Biology, Western Reserve University, *Adelbert College, Cleveland, Ohio.*

HESS, WALTER N., A.B. (Oberlin), A.M., Ph.D. (Cornell), Professor of Biology, De Pauw University, *Greencastle, Indiana.*

HICKERNELL, L. B., A.B., A.M., Ph.D. (Princeton), Assistant Professor of Zoölogy, Syracuse University, *1052 Ackerman Ave., Syracuse, N. Y.*

HILTON, WILLIAM ATWOOD, B.S., Ph.D. (Cornell), Professor of Zoölogy, Pomona College, Claremont, California; Director Laguna Marine Biological Laboratory; Editor, Journal of Entomology and Zoölogy, *Claremont, Calif.*

HOGUE, MARY JANE, A.B. (Goucher), Ph.D. (Würzburg), Fellow in Protozoölogy, School of Hygiene and Public Health, *Johns Hopkins University, 821 Charles St., Baltimore, Md.*

HOLMES, SAMUEL J., B.S., M.S. (California), Ph.D. (Chicago), Professor of Zoölogy, *University of California, Berkeley, Calif.*

HOOKER, DAVENPORT, B.A., M.A., Ph.D. (Yale), Professor of Anatomy, University of Pittsburgh, School of Medicine, *University of Pittsburgh, Pittsburgh, Pa.*

HOUSER, GILBERT LOGAN, B.S., M.S. (Iowa), Ph.D. (Johns Hopkins), Professor of Animal Biology and Director of the Laboratories of Animal Biology, State University of Iowa, *430 Iowa Avenue, Iowa City, Iowa.*

HOWARD, A. D., B.S., (Amherst), M. S. (Northwestern), Ph.D. (Harvard), Scientific Assistant, United States Bureau of Fisheries, Fairport Biological Laboratory, *United States Biological Laboratory, Fairport, Iowa.*

HUNT, HARRISON RANDALL, B.S., Allegheny College, M.A., Ph.D. (Harvard), Professor of Zoölogy, University of Mississippi, *Oxford, Miss.*

HUNTSMAN, ARCHIBALD GOWANLOCK, B.A., (M.B. Toronto), F.R.S.C. (Canada), Professor of Marine Biology, Biological Department, *University of Toronto, Toronto, Canada.*

HUSSAKOF, LOUIS, B.S. (College of the City of New York), Ph.D. (Columbia), Curator of Ichthyology, American Museum of Natural History, *1537 50th Street, Borough Park, Brooklyn, N. Y.*

HUXLEY, JULIAN SORELL, B.A. (Oxford), Senior Demonstrator, Department of Zoölogy and Comparative Anatomy, Fellow of New College, Oxford, *The Museum, Oxford, England.*

HYDE, ROSCOE RAYMOND, A.B., A.M. (Indiana), Ph.D. (Columbia), Associate in Immunology, School of Hygiene and Public Health, *Johns Hopkins University, 310–312 W. Monument St., Baltimore, Maryland.*

HYMAN, L. H., Ph.D. (Chicago), Research Assistant, University of Chicago, *Hull Zoölogical Laboratory, University of Chicago, Chicago, Illinois.*

IBSEN, HEMAN LAWRITZ, B.S., M.S., Ph.D. (Wisconsin), Professor of Genetics, Department of Animal Husbandry, *Kansas State Agricultural College, Manhattan, Kan.*

ISELY, FREDERICK B., B.S. (Fairmount), M.S. (Chicago), Professor of Biology, *Culver-Stockton College, Canton, Mo.*

JACOBS, MERKEL HENRY, A.B., Ph.D. (Pennsylvania), Assistant Professor of Zoölogy, Zoölogical Laboratory, *University of Pennsylvania, Philadelphia, Pa.*

JENNINGS, HERBERT S., B.S. (Michigan), A.M., Ph.D. (Harvard), LL.D. (Clark), S.D. (Michigan), Henry Walters Professor of Zoölogy and Director of the Zoölogical Laboratory, *Johns Hopkins University, Baltimore, Md.*

JEWELL, MINNA E., A.B. (Colorado College), A.M., Ph.D. (Illinois), Assistant Professor of Zoölogy, *Milwaukee-Downer College, Milwaukee, Wisconsin.*

JOB, THESLE T., A.B., A.M., Ph.D., Associate Professor of Anatomy, Loyola University School of Medicine, *706 S. Lincoln St., Chicago, Ill.*

JOHANNSEN, OSKAR AUGUSTUS, B.S. (Illinois), A.M. (Cornell), Ph.D. (Cornell), Professor of Entomology, Cornell University, College of Agriculture, *234 Parkway, Ithaca, N. Y.*

JOHNSON, CHARLES EUGENE, B.A., M.A., Ph.D. (Minnesota) Assistant Professor of Zoölogy, University of Kansas, *616 West 17th Street, Lawrence, Kansas.*

JOHNSTON, JOHN B., Ph.D. (Michigan), Professor of Comparative Neurology, University of Minnesota, *Minneapolis, Minn.*

JORDAN, HARVEY ERNEST, B.A., M.A. (Lehigh), Ph.D. (Princeton), Professor of Histology and Embryology, *University of Virginia, Charlottesville, Va.*

JUDAY, CHANCEY, A.B., A.M. (Indiana), Biologist, Wisconsin Geological and Natural History Survey; Lecturer in Zoölogy, *University of Wisconsin, Madison, Wisconsin.*

JUST, ERNEST EVERETT, Ph.D. (Chicago), Professor of Zoölogy, *Howard University, Washington, D. C., 412 T Street, Washington, D. C.*

KAMPMEIER, OTTO F., B.A. (Iowa), Ph.D. (Princeton), Professor and Director of the Department of Anatomy, Marquette School of Medicine, *4th and Reservoir Sts., Milwaukee, Wis.*

KEPNER, WILLIAM ALLISON, A.B., A.M. (Franklin and Marshall College, Lancaster, Pa.), Ph.D. (Virginia), Associate Professor of Biology, University of Virginia, *University, Va.*

KINCAID, TREVOR, B.S., M.A. (University of Washington), Head of Department of Zoölogy, *University of Washington, Seattle, Wash.*

KINDRED, JAMES ERNEST, A.B., A.M., Ph.D. (Illinois), Instructor in Biology, *Western Reserve Dental School, Cleveland, Ohio, 1353 E. 9th St., Cleveland, Ohio.*

KING, HELEN DEAN, A.B. (Vassar), A.M., Ph.D. (Bryn Mawr), Assistant Professor of Embryology, The Wistar Institute of Anatomy and Biology, *The Wistar Institute, Thirty-sixth St. and Woodland Ave., West Philadelphia, Pa.*

KINGSBURY, BENJAMIN FREEMAN, Ph.D. (Cornell), M.D. (Freiburg), Professor of Histology and Embryology, Cornell University, *2 S. Avenue, Ithaca, N. Y.*

KINGSLEY, JOHN STERLING, A.B. (Williams), Sc.D. (Princeton), Professor of Zoölogy, *University of Illinois, Urbana, Ill.*

KIRKHAM, WILLIAM BARRI, B.A., M.A., Ph.D. (Yale), Assistant Professor of Biology, Sheffield Scientific School, Yale University, *103 Everit Street, New Haven, Conn.*

KNOWER, HENRY McE., A.B., Ph.D. (Johns Hopkins), Professor of Anatomy, Medical Department, *University of Cincinnati, Cincinnati, Ohio.*

KOFOID, CHARLES ATWOOD, A.B., Sc.D. (Hon.) (Oberlin), A.M., Ph. D. (Harvard), Sc.D. (Hon.) (University of Wales). Professor of Zoölogy and Assistant Director of the Scripps Institution for Biological Research, *University of California, Berkeley, Calif.*

KORNHAUSER, SIDNEY, A.B. (Pittsburgh), A.M. Ph.D. (Harvard), Professor of Zoölogy, *Denison University, Granville, O.*

KRECKER, FREDERIC H., A.B., Ph.D. (Princeton), A.M. (Cornell), Assistant Professor of Zoölogy, *Ohio State University, Columbus, Ohio.*

KRIBS, HERBERT GUY, A.B. (Oberlin), Ph.D. (Pennsylvania), B.A. (Union), Assistant in Zoölogy, *University of Pennsylvania, Philadelphia, Pa.*

KUDO, ROKUSABURO, D.Ag.Sc. (Tokio), Instructor in Zoölogy, *University of Illinois, Urbana, Illinois.*

KUNKEL, BEVERLY WAUGH, Ph.B., Ph.D. (Yale), Professor of Biology, *Lafayette College, Easton, Pa.*

KUNTZ, ALBERT, B.A. (Morningside), Ph.D. (State University of Iowa), M.D. (St. Louis University School of Medicine), Professor of Anatomy and Biology, *St. Louis University School of Medicine, 1402 S. Grand Ave., St. Louis, Mo.*

LANDACRE, FRANCIS LEROY, A.B. (Ohio), Ph.D. (Chicago), Professor of Anatomy, *Ohio State University, Columbus, Ohio.*

LANE, HENRY HIGGINS, Ph.B. (DePauw), A.M. (Indiana), Ph.D. (Princeton), Professor of Zoölogy, *Phillips University, East Enid, Oklahoma.*

LA RUE, GEORGE R., B.S. (Doane), A.M. (Nebraska), Ph.D. (Illinois), Assistant Professor of Zoölogy, *University of Michigan, Ann Arbor, Mich.*

LASHLEY, KARL SPENCER, A.B. (West Virginia), M.S. (Pittsburgh), Ph.D. (Johns Hopkins), Assistant Professor of Psychology, *University of Minnesota, Minneapolis, Minn.*

LAURENS, HENRY, A.M. (Charleston), Ph.D. (Harvard), Assistant Professor of Biology, Yale College, Osborn Zoölogical Laboratory, *Yale University, New Haven, Conn.*

LEE, THOMAS G., B.S., M.D. (Pennsylvania), Professor of Comparative Anatomy, *University of Minnesota, Institute of Anatomy, Minneapolis, Minn.*

LEFEVRE, GEORGE, A.B., Ph.D. (Johns Hopkins), Professor of Zoölogy, *University of Missouri, Columbia, Mo.*

LILLIE, FRANK R., B.A. (Toronto), Ph.D. (Chicago), Professor of Embryology and Chairman of the Department of Zoölogy, University of Chicago; Director, Marine Biological Laboratory, Woods Hole, Mass., *University of Chicago, Chicago, Ill.*

LILLIE, RALPH S., A.B. (Toronto), Ph.D. (Chicago), Biologist, Nela Research Laboratories, Department of Pure Science, *Nela Research Laboratories, Nela Park, Cleveland, Ohio.*

LINTON, EDWIN, A.B., S.M. (Washington and Jefferson), Ph.D. (Yale), 1408 Rosemary Lane, Columbia, Mo.

LIPPINCOTT, WILLIAM ADAMS, A.B. (Illinois College), S.B. (Iowa State), S.M. Ph.D., (Wisconsin), Professor of Poultry Husbandry and Poultry Husbandman, Kansas State Agricultural College, *Manhattan, Kansas.*

LOCY, WILLIAM ALBERT, Ph.D. (Chicago), Sc.D. (Hon.) (Michigan), Professor of Zoölogy and Director of the Zoölogical Laboratory, Northwestern University, *1745 Arrington Ave., Evanston, Ill.*

LONG, JOSEPH A., S.B., A.M., Ph.D. (Harvard), Assistant Professor of Embryology, University of California, *1534 La Loma Avenue, Berkeley, Cal.*

LONGLEY, WILLIAM, M.A., Ph.D. (Yale), Professor of Biology, *Goucher College, Baltimore, Md.*

LUND, ELMER J., Ph.D. (Johns Hopkins University), Assistant Professor of Zoölogy, *University of Minnesota, Minneapolis, Minn.*

LUTZ, FRANK E., A.B. (Haverford), A.M., Ph.D. (Chicago), Associate Curator of Invertebrate Zoölogy, American Museum of Natural History, *77th Street and Central Park West, New York City.*

LYNCH, RUTH STOCKING (Mrs.), A.B. (Goucher), Ph.D. (Johns Hopkins), Independent Research, *The Johns Hopkins Medical School, 3A Seville Apartments, Lake Drive, Baltimore, Md.*

MCCLUNG, C. E., Ph.G., A.B., A.M., Ph.D. (Kansas), Professor of Zoölogy, University of Pennsylvania, Chairman Division of Biology and Agriculture, *National Research Council, Washington, D. C., or Zoölogical Laboratory, University of Pennsylvania, Philadelphia, Pa.*

McClure, Charles F. W., A.B., A.M. (Princeton), D.Sc. (Columbia), Professor of Zoölogy, *Princeton University, Princeton, N. J.*

McCulloch, Irene, Ph.D. (California), Professor of Biology, Sophie Newcomb College, *New Orleans, La.*

MacCurdy, Hansforth M., A.B. (Ohio Wesleyan), A.M., Ph.D. (Harvard), Professor of Biology, Alma College, *701 Center St., Alma, Mich.*

MacDowell, Edwin Carleton, A.B. (Swarthmore), S.M., Sc.D. (Harvard), Research Investigator, Station for Experimental Evolution, Carnegie Institution of Washington, *Cold Spring Harbor, Long Island, N. Y.*

MacGillivray, Alexander Dyer, Ph.D. (Cornell), Associate Professor Systematic Entomology, University of Illinois, *603 West Michigan Ave., Urbana, Ill.*

McGregor, James Howard, B.S. (Ohio State University), M.A., Ph.D. (Columbia), Associate Professor of Zoölogy, *Columbia University, New York City.*

McIndoo, Norman Eugene, A.B., A.M. (Indiana), Ph.D. (Pennsylvania), Insect Physiologist, *Bureau of Entomology, Washington, D. C.*

Magath, Thomas Byrd, Ph.B. (Emory University), M.S. (Millikin University), Ph.D. (University of Illinois), M.D. (University of Illinois), Parasitologist, *Mayo Clinic, Rochester, Minn.*

Mark, Edward L., A.B. (Michigan), Ph.D. (Leipzig), LL.D. (Michigan), LL.D. (Wisconsin), Hersey Professor of Anatomy and Director of the Zoölogical Laboratory, Harvard University, *109 Irving Street, Cambridge, Mass.*

Marshall, Ruth, B.S. , M.S. (Wisconsin), Ph.D. (Nebraska), Professor of Biology, *Rockford College, Rockford, Ill.*

Marshall, William Stanley, B.S. (Swarthmore), Ph.D. (Leipzig), Associate Professor of Entomology, University of Wisconsin, *139 East Gilman Street, Madison, Wisconsin.*

Mast, Samuel Ottmar, B.S. (Michigan), Ph.D. (Harvard), M.Pd. (Michigan Normal College), Professor of Zoölogy, *Johns Hopkins University, Baltimore, Md.*

Mavor, James Watt, B.A. (Cantab.), Ph.D. (Harvard), Assistant Professor of Zoölogy, *Union College, Schenectady, N. Y.*

May, Henry G., S.B. (Rochester), Ph.D. (Illinois), Professor of Bacteriology Rhode Island State College and Chief, Division of Animal Breeding and Pathology, Agr. Exp. Station, *Kingston, R. I.*

Mead, Albert Davis, A.B. (Middlebury), A.M. (Brown), Ph.D. (Chicago), Sc.D. (Pittsburgh), Professor of Biology, Brown University, *283 Wayland Avenue, Providence, R. I.*

Metz, Charles W., B.A. (Pomona), Ph.D. (Columbia), Station for Experimental Evolution, Carnegie Institution of Washington, *Cold Spring Harbor, Long Island, N. Y.*

Meyer, Arthur William. B.S. (Wisconsin), M.D. (Johns Hopkins), Professor of Anatomy, Stanford Jr. University, *121 Waverly Street, Palo Alto, Cal.*

Middleton, Austin Ralph, A.B., Ph.D. (Johns Hopkins), Professor of Zoölogy, Director of Department of Biology, *University of Louisville, 1st and Chestnut Sts., Louisville, Ky.*

Minnich, Dwight Elmer, A.B. (Miami), Ph.D. (Harvard), Instructor in Animal Biology, *University of Minnesota, Minneapolis.*

MOENKHAUS, WILLIAM J., A.B. (Indiana), Ph.D. (Chicago), Professor of Physiology, Indiana University, *501 Fess Avenue, Bloomington, Ind.*

MOODY, JULIA ELEANOR, B.S., M.A. (Mt. Holyoke), Ph.D. (Columbia), Associate Professor of Zoölogy, Wellesley College, *15 Appleby Road, Wellesley, Mass.*

MOORE, CARL R., B.S., A.M. (Drury), Ph.D. (Chicago), Instructor in Zoölogy, *University of Chicago, Chicago, Ill.*

MORGAN, ANN HAVEN, A.B., Ph.D. (Cornell), Professor of Zoölogy, *Mt. Holyoke College, South Hadley, Mass.*

MORGAN, THOMAS HUNT, B.S. (Kentucky), Ph.D. (Johns Hopkins), Professor of Experimental Zoölogy, *Columbia University, New York City.*

MORGULIS, SERGIUS, A.M. (Columbia), Ph.D. (Harvard), Professor of Biochemistry and Physiology, *Creighton University Medical School, Omaha, Neb.*

MORRILL, ALBRO DAVID, B.S., M.S. (Dartmouth), Professor of Biology, *Hamilton College, Clinton, Oneida County, N. Y.*

MORRILL, CHARLES V., A.M., Ph.D. (Columbia), Assistant Professor of Anatomy, Cornell University Medical College, *28th Street and First Avenue, New York City.*

MOSHER, EDNA, B.S. (Cornell), Ph.D. (Illinois), Acting Professor of Biology, *University of New Mexico, Albuquerque, N. Mex.*

MULLENIX, ROLLIN CLARKE, A.B., A.M. (Wheaton), Ph.D. (Harvard), Professor of Zoölogy, Lawrence College, *461 Washington St., Appleton, Wisconsin.*

MULLER, HERMAN J., A.B., A.M., Ph.D. (Columbia), Dept. of Zoölogy, Univ. of Texas, Austin, Texas.

MUTTKOWSKI, RICHARD ANTHONY, A.B., A.M., Ph.D. (Wisconsin), Assistant Professor of Zoölogy and Entomology, University of Idaho, *Moscow, Idaho.*

NABOURS, ROBERT K., Ph.D. (Chicago), Professor of Zoölogy and Experiment Station Zoölogist, *Kansas Agricultural College, Manhattan, Kansas.*

NACHTRIEB, HENRY FRANCIS, B.S. (Minnesota), Professor of Animal Biology and Head of the Department, *University of Minnesota, Minneapolis, Minn.*

NEAL, HERBERT VINCENT, A.B., A.M., Ph.D. (Harvard), Professor of Zoölogy, Tufts College, *57 Midford Hillside, Tufts College, Mass.*

NELSON, JAMES ALLEN, Ph.B. (Kenyon College), Ph.D. (Pennsylvania), Collaborator Bureau of Entomology, U. S. Department of Agriculture, *Route 3, Mount Vernon, Ohio.*

NELSON, THURLOW, CHASE B.A. (Rutgers), Ph.D. (Wisconsin), Asst. Professor of Zoölogy, Rutgers College, Biologist, State Board of Shell Fisheries, *855 Adelaide Ave., New Brunswick, N. J.*

NEWMAN, HORATIO HACKETT, B.A., (McMaster), Ph.D. (Chicago), Professor of Zoölogy and Dean in the College of Science, *University of Chicago, Chicago, Ill.*

NORRIS, HARRY WALDO, A.B., A.M. (Grinnell), Professor of Zoölogy, *Grinnell College, Grinnell, Iowa.*

NOWLIN, NADINE (Miss), A.B., A.M. (Kansas), Assistant Professor of Zoölogy, *University of Kansas, 1144 Louisiana St., Lawrence, Kansas.*

NUTTING, C. C., A.B., A.M. (Blackburn University), Professor of Zoölogy, *State University of Iowa, Iowa City, Iowa.*

O'DONOGHUE, CHARLES H., D.Sc. (London), F.Z.S., Professor of Zoölogy, *University of Manitoba, Winnipeg, Canada.*

Olmsted, J. M. D., Ph.D. (Harvard), Instructor in Physiology, Toronto University, *Toronto, Canada.*

Osborn, Henry Fairfield, A.B., Sc.D. (Princeton), LL.D. (Hon.) (Trinity, Princeton, Columbia), D.Sc. (Hon.) (Cambridge University), Ph.D. (Hon.) (University of Christiana, Upsala); Research Professor of Zoölogy, Columbia; President Board of Trustees, American Museum Natural History; Curator Emeritus, Department of Vertebrate Paleontology, Vertebrate Paleontologist, United States Geological Survey; American Museum of Natural History, *Seventy-seventh Street and Central Park West, New York City.*

Osborn, Henry Leslie, A.B. (Wesleyan), Ph.D. (Johns Hopkins), Professor of Biology, Hamline University, *1500 Hewitt Avenue, St. Paul, Minn.*

Osborn, Herbert, B.Sc., M.Sc., D.Sc. (Iowa State College), Research Professor Ohio State University, *1952 Concord Road, Upper Arlington, Columbus, Ohio.*

Osburn, Raymond C., Ph.D. (Columbia), Professor of Zoölogy, *Ohio State University, Columbus, Ohio.*

Packard, Charles, M.S., Ph.D., *Peking Union Medical College, Peking, China.*

Painter, Theophilus Shickel, A.B., (Roanoke) A.M., Ph.D. (Yale), Adjunct Professor of Zoölogy, *University of Texas, Austin, Texas.*

Parker, George Howard, S.B., Sc.D. (Harvard), Professor of Zoölogy, Harvard University, *16 Berkeley Street, Cambridge, Mass.*

Parshley, H. M., A.B., A.M., Sc.D. (Harvard), Associate Professor of Zoölogy, Smith College, *Department of Zoölogy, Smith College, Northampton, Mass.*

Patten, Bradley Merrill, A.B. (Dartmouth), A.M., Ph.D.. (Harvard), Assistant Professor of Histology and Embryology, Western Reserve University Medical School, *1353 East 9th Street, Cleveland, Ohio.*

Patten, William, B.S., (Harvard) M.A., Ph.D. (Leipzig), Professor of Zoölogy, *Dartmouth College, Hanover, N. H.*

Patterson, John Thomas, B.S. (Wooster), Ph.D. (Chicago), Professor of Zoölogy, University of Texas, *University Station, Austin, Texas.*

Payne, Fernandus, A.B., A.M. (Indiana), Ph.D. (Columbia), Professor of Zoölogy, Indiana University, *Bloomington, Indiana.*

Pearl, Raymond, A.B. (Dartmouth), Ph.D. (Michigan), D.S. (Dartmouth), LL.D. (Maine), Professor of Biometry and Vital Statistics, School of Hygiene and Public Health, *Johns Hopkins University, Baltimore, Md.*

Pearse, Arthur Sperry, B.S., A.M. (Nebraska), Ph.D. (Harvard), Professor of Zoölogy, *University of Wisconsin, Madison, Wis.*

Peebles, Florence, A.B. (Goucher), Ph.D. (Bryn Mawr), Associate Professor of Physiology, Bryn Mawr College, *Bryn Mawr, Pa.*

Perkins, Henry F., A.B. (Vermont), Ph.D. (Johns Hopkins), Professor of Zoölogy, University of Vermont, *205 South Prospect Street, Burlington, Vt.*

Petrunkevitch, Alexander, Ph.D. (Freiburg), Assistant Professor of Zoölogy, *Sheffield Scientific School Zoölogical Laboratory, Yale University, New Haven, Conn.*

Phillips, Everett Franklin, A.B. (Allegheny), Ph.D. (Pennsylvania), Agriculturalist, Bureau of Entomology, *United States Department of Agriculture, Washington, D. C.*

Piersol, George Arthur, M.S. (Pennsylvania), Sc.D. (Pennsylvania College), Professor of Anatomy, University of Pennsylvania, *4724 Chester Avenue, Philadelphia, Pa.*

PIKE, FRANK H., A. B., (Indiana) Ph.D. (Chicago), Associate Professor of Physiology, Columbia University, *437 West 59th Street, New York City.*

POWERS, EDWIN B., Instructor in Zoölogy, *University of Nebraska, Lincoln, Neb.*

PRATT, HENRY SHERRING, A.B. (Michigan), A.M., Ph.D. (Leipzig), Professor of Biology, *Haverford College, Haverford, Pa.*

RAND, HERBERT WILBUR, A.B. (Allegheny, Harvard), A.M., Ph.D. (Harvard), Associate Professor of Zoölogy, Harvard University, *Museum of Comparative Zoölogy, Cambridge, Mass.*

RANSOM, BRAYTON HOWARD, B.Sc., M.A., Ph.D. (Nebraska), Chief Zoölogical Division, Bureau Animal Industry, United States Department of Agriculture, *Bureau of Animal Industry, Washington, D. C.*

REED, HUGH DANIEL, B.S., Ph.D. (Cornell), Professor of Zoölogy, Cornell, *McGraw Hall, Ithaca, N. Y.*

REESE, ALBERT MOORE, A.B., Ph.D. (Johns Hopkins), Professor of Zoölogy, *West Virginia University, Morgantown, W. Va.*

REIGHARD, JACOB ELLSWORTH, Ph.B. (Michigan), Professor of Zoölogy, Director of Zoölogical Laboratory and Biological Station, *University of Michigan, Ann Arbor, Michigan.*

REINKE, EDWIN EUSTACE, M.A. (Lehigh), Ph.D. (Princeton), Assistant Professor of Biology, *Vanderbilt University, Nashville, Tenn.*

RICE, EDWARD LORANUS, A.B. (Wesleyan), Ph.D. (Munich), Professor of Zoölogy, *Ohio Wesleyan University, Delaware, Ohio.*

RICHARDS, A., B.A. (Kansas), M.A. (Wisconsin), Ph.D. (Princeton), Professor of Zoölogy, Dept. of Zoölogy, Univ. of Oklahoma, Norman, Okla.

RICHARDS, MILDRED H., A.B. (Goucher), A.M., Ph.D. (Columbia), Dept. of Zoölogy, Univ. of Oklahoma, Norman, Okla.

RIDDLE, OSCAR, A.B. (Indiana), Ph.D. (Chicago), Resident Investigator Carnegie Institution of Washington, *Cold Spring Harbor, Long Island, N. Y.*

RILEY, WILLIAM ALBERT, B.S. (Depauw), Ph.D. (Cornell), Professor of Entomology, University of Minnesota, *University Farm, St. Paul, Minnesota.*

RITTER, WILLIAM E., B.S. (California), Ph.D. (Harvard), Director of Scripps Institution of Biological Research of the University of California, Professor of Zoölogy, University of California, *La Jolla, Calif.*

ROBERTS, ELMER, B.S., Ph.D. (University of Illinois), Associate in Genetics, University of Illinois, *College of Agriculture, Urbana, Ill.*

ROBERTSON, ALBERT DUNCAN, B.A. (Toronto), Professor and Head of Department of Biology, Western University, *Dept. of Biology, Western University, London, Ont., Canada.*

ROBERTSON, ALICE, B.S., M.S., Ph.D. (California), Assistant Professor of Zoölogy, *Wellesley College, Wellesley, Mass.*

ROBERTSON, W. REES BREBNER, A.B. (Kansas), Ph.D. (Harvard), Associate Professor of Zoölogy, University of Kansas, *1701 Louisiana Street, Lawrence, Kansas.*

ROGERS, CHARLES GARDNER, A.B., A.M. (Syracuse), Ph.D. (California), Professor of Comparative Physiology, Oberlin College, *378 Reamer Place, Oberlin, Ohio.*

ROGERS, FRED TERRY, A.B., Ph.D. (Chicago), Professor of Physiology, Baylor Medical College, Dallas, Texas.

ROOT, FRANCIS METCALF, A.B., A.M. (Oberlin), Ph.D. (Johns Hopkins), Teaching Fellow in Medical Entomology, *School of Hygiene and Public Health, Johns Hopkins University, 310–312 West Monument St., Baltimore, Md.*

RUTHVEN, ALEXANDER G., B.S. (Morningside), Ph.D. (Michigan), Director Museum of Zoölogy, Professor of Zoölogy, University of Michigan, *Museum of Zoölogy, Ann Arbor, Mich.*

SCHAEFFER, ASA ARTHUR, A.B. (Franklin and Marshall), Ph.D. (Johns Hopkins), Professor of Zoölogy, *University of Tennessee, Knoxville, Tenn.*

SCHIEDT, RICHARD C. F., Ph.D. (Pennsylvania), Sc.D. (Hon.) (Franklin and Marshall), *Lancaster, Pa.*

SCOTT, JOHN W., A.B., A.M. (Missouri), Ph.D. (Chicago), Professor of Zoölogy, Research Parasitologist, *University of Wyoming, Laramie, Wyoming.*

SCOTT, GEORGE G., A.B., A.M. (Williams), Ph.D. (Columbia), Chairman Department of Biology, *College of the City of New York, New York City.*

SCOTT, WILL, Ph.D. (Indiana), Associate Professor of Zöology, *Indiana University, Bloomington, Indiana.*

SHELFORD, VICTOR ERNEST, B.S., Ph.D. (Chicago), Associate Professor of Zoölogy, University of Illinois, and Biologist in charge of Research Laboratories, Natural History Survey, *The Vivarium, Wright and Healy Sts., Champaign, Illinois.*

SHOREY, MARIAN LYDIA, A.M., Ph.B. (Brown), Ph.D. (Chicago), *Huguenot College; Wellington, South Africa.*

SHULL, AARON FRANKLIN, A.B. (Michigan), Ph.D. (Columbia), Associate Professor of Zoölogy, University of Michigan, *520 Linden Street, Ann Arbor, Mich.*

SIGERFOOS, CHARLES P., B.S. (Ohio State), Ph.D. (Johns Hopkins), Professor of Zoölogy, *University of Minnesota, Minneapolis, Minn.*

SMALLWOOD, WILLIAM MARTIN, Ph.D. (Harvard), Professor of Comparative Anatomy, Syracuse University, *525 Euclid Avenue, Syracuse, N Y.*

SMITH, BERTRAM GARNER, A.B. (Michigan), Ph.D. (Columbia), Associate Professor of Zoölogy, Michigan State Normal College, *122 College Place, Ypsilanti, Mich.*

SMITH, ELIZABETH ANITA, A.B. (Cincinnati), M.A., Ph.D. (Wisconsin), Assistant Professor, *University of Wisconsin, Biology Bldg., Madison, Wisconsin.*

SMITH, FRANK, Ph.B. (Hillsdale College), A.M. (Harvard), Professor of Systematic Zoölogy, *University of Illinois, Urbana, Ill.*

SNYDER, THOMAS ELLIOTT, A.B. (Columbia), M.F. (Yale), Ph.D. (George Washington), Specialist in Forest Entomology, *Bureay of Entomology, U. S. Department of Agriculture, Washington, D. C.*

STOCKARD, CHARLES RUPERT, B.S., M.S. (Mississippi Agricultural and Mechanical College), Ph.D. (Columbia), Professor of Anatomy, Cornell University Medical School, *First Avenue and Twenty-eighth Street, New York City.*

STREETER, GEORGE L., A.B. (Union), A.M., M.D. (Columbia), Director of Embryology, Carnegie Institution, *Johns Hopkins Medical School, Baltimore, Md.*

STROMSTEN, FRANK ALBERT, B.S., M.S. (Iowa), D.Sc. (Princeton), Associate Professor of Animal Biology, State University of Iowa, *943 Iowa Ave., Iowa City, Iowa.*

STRONG, OLIVER S., A.B., A.M. (Princeton), Ph.D. (Columbia), Associate Professor in Neurology, Columbia University, College of Physicians and Surgeons, *437 West Fifty-ninth Street, New York City.*

STRONG, REUBEN MYRON, A.B. (Oberlin), M.A., Ph.D. (Harvard), Professor of Anatomy, Loyola University School of Medicine, *706 South Lincoln Street, Chicago, Ill.*

STURTEVANT, ALFRED H., A.B., Ph.D. (Columbia), Stanford Univ., Calif.

SUMNER, FRANCIS B., B.S. (Minnesota), Ph.D. (Columbia), Biologist, Scripps Institution for Biological Research, *La Jolla, Calif.*

SURFACE, FRANK M., A.B., A.M. (Ohio State), Ph.D. (Pennsylvania), Biologist, *The Washington Herald, Washington, D. C.*

SWEZY, OLIVE, B.S., M.S., Ph.D. (California), Associate in Zoölogy, Assistant Zoölogist, Scripps Institution for Biological Research, University of California, *East Hall, University of California, Berkeley, Calif.*

SWINGLE, WILBUR WILLIS, A.B., A.M. (Kansas), Ph.D. (Princeton), Instructor in Biology, Sheffield Scientific School, Yale University, *Osborn Zoological Laboratory, New Haven, Conn.*

TALIAFERRO, WILLIAM HAY, B.S. (University of Virginia), Ph.D. (Johns Hopkins), Instructor in Protozoölogy, Johns Hopkins University, School of Hygiene and Public Health, *310–312 W. Monument St., Baltimore, Md.*

TANNREUTHER, GEORGE W., A.B. (Manchester), A.M. (Antioch), Ph.D. (Chicago), Instructor in Zoölogy, *University of Missouri, Columbia, Mo.*

TASHIRO, SHIRO, B.S., Ph.D. (Chicago), Associate Professor of Biological Chemistry, University of Cincinnati, *College of Medicine, Cincinnati, Ohio.*

TAYLOR, CHARLES VINCENT, Ph.D. (California), Assistant Professor of Zoölogy, University of California, *East Hall, University of California, Berkeley, Calif.*

TENNENT, DAVID HILT, B.S. (Olivet), Ph.D. (Johns Hopkins), Professor of Biology, *Bryn Mawr College, Bryn Mawr, Pa.*

THOMPSON, CAROLINE BURLING, S.B., Ph.D. (Pennsylvania), Associate Professor of Zoölogy, *Wellesley College, Wellesley, Mass.*

TORREY, HARRY BEAL, B.S., M.S. (California), Ph.D. (Columbia), Professor of Zoölogy, *University of Oregon, Eugene, Ore.*

TREADWELL, AARON L., B.S., M.S. (Wesleyan), Ph.D. (Chicago), Professor of Biology, *Vassar College, Poughkeepsie, N. Y.*

TURNER, CLARENCE LESTER, A.B., A.M. (Ohio Wesleyan), Ph.D. (Wisconsin), Professor of Zoölogy, Beloit College, *Beloit, Wisconsin.*

VAN CLEAVE, HARLEY JONES, B.S. (Knox College), M.S., Ph.D. (Illinois), Assistant Professor of Zoölogy, *300 Natural History Building, University of Illinois, Urbana, Ill.*

WAGNER, GEORGE, B.A. (Kansas), M.A. (Michigan), Associate Professor of Zoölogy, *University of Wisconsin, Biology Building, Madison, Wis.*

WAITE, FREDERICK CLAYTON, Litt.B. (Adelbert), A.M. (Western Reserve), A.M., Ph.D. (Harvard), Professor of Histology and Embryology, School of Medicine, Western Reserve University, *1353 East 9th Street, Cleveland, Ohio.*

WALTER, HERBERT EUGENE, A.B. (Bates), A.M. (Brown), Ph.D. (Harvard), Associate Professor of Biology, *Brown University, Providence, R. I.*

WARD, HENRY BALDWIN, A.B. (Williams), A.M., Ph.D. (Harvard), Professor of Zoölogy, *University of Illinois, Urbana, Illinois.*

WELCH, PAUL SMITH, A.B. (James Millikin), A.M., Ph.D. (Illinois), Assistant Professor of Zoölogy, *University of Michigan, Ann Arbor, Mich.*

WEESE, ASA ORRIN, A.B. (Minnesota), A.M. (Illinois), Professor of Biology, University of New Mexico, Secretary, Ecological Society of America, *Albuquerque, N. Mex.*

WELLS, MORRIS MILLER, B.S. (Chicago), Ph.D. (Illinois), President General Biological Supply Co., *5508 Kimbark Ave., Chicago, Ill.*

WENRICH, DAVID HENRY, B.A., M.A., Ph.D., Instructor in Zoölogy, *University of Pennsylvania, Zoölogical Laboratory, Philadelphia, Pa.*

WENTWORTH, EDWARD N., M.S. (Iowa), Professor of Animal Husbandry, *Kansas State Agricultural College, Manhattan, Kan.*

WHEELER, WILLIAM MORTON, Ph.D. (Clark), Professor of Economic Entomology, *Bussey Institution, Forest Hills, Boston, Mass.*

WHITE, GERTRUDE MAREAN, B.A. (Milwaukee-Downer), M.A., Ph.D. (Wisconsin), Instructor in Biology, *Margaret Morrison Division, Carnegie Institute of Technology, Pittsburgh, Pa.*

WHITING, PHINEAS W., A.B., M.S., Ph.D., Professor of Biology, *St. Stephen's College, Annandale-on-Hudson, N. Y.*

WHITNEY, DAVID DAY, B.A. (Wesleyan), M.A., Ph.D. (Columbia), Professor of Zoölogy, *University of Nebraska, Lincoln, Neb.*

WIEMAN, HARRY LEWIS, A.B., A.M. (Cincinnati), Ph.D. (Chicago), Professor of Zoölogy, *University of Cincinnati, Cincinnati, Ohio.*

WILDER, HARRIS HAWTHORNE, A.B. (Amherst), Ph.D. (Freiburg), Professor of Zoölogy, *Smith College, Northampton, Mass.*

WILDER, INEZ WHIPPLE, Ph.B. (Brown), M.A. (Smith), Associate Professor of Zoölogy, Smith College, *27 Belmont Ave., Northampton, Mass.*

WILDMAN, EDWARD E., B.S., M.S., Ph.D. (Pennsylvania), Head Department of Science, West Philadelphia High School for Girls, Instructor in Zoölogy, University of Pennsylvania, *4331 Osage Avenue, Philadelphia, Pa.*

WILLARD, W. A., Ph.B. (Grinnell), A.M. (Tufts and Harvard), Ph.D. (Harvard), Professor of Anatomy, *University of Nebraska, College of Medicine, Omaha, Neb.*

WILLIAMS, STEPHEN RIGGS, A.B., A.M. (Oberlin), A.M., Ph.D. (Harvard), Professor of Zoölogy and Geology, Miami University, *300 East Church Street, Oxford, Ohio.*

WILSON, EDMUND B., Ph.B. (Yale), Ph.D. (Johns Hopkins), LL.D. (Yale, Chicago, Hopkins), M.D. (Hon.) (Leipzig), Sc.D. (Cambridge), Da Costa Professor of Zoölogy, *Columbia University, New York City.*

WILSON, HENRY VAN PETERS, A.B., Ph.D. (Johns Hopkins), Professor of Zoölogy, *University of North Carolina, Chapel Hill, N. C.*

WODSEDALEK, JERRY EDWARD, Ph.B., M. Ph., Ph.D. (Wisconsin), Professor of Zoölogy and Head of the Department of Zoölogy and Entomology, *University of Idaho, Moscow, Idaho.*

WOLCOTT, ROBERT HENRY, B.S., M.D. (Michigan), A.M. (Nebraska), Professor and Head of the Department of Zoölogy, *University of Nebraska, Lincoln, Neb.*

WOODRUFF, LORANDE LOSS, A.B., A.M., Ph.D. (Columbia), M.A. (Yale), Professor of Biology, Yale University, *Osborn Zoölogical Laboratory, New Haven, Conn.*

WRIGHT, ALBERT HAZEN, A.B., A.M., Ph.D. (Cornell), Assistant Professor of Zoölogy, Cornell University, *Upland Road, Cayuga Hts., Ithaca, N. Y.*

WRIGHT, SEWALL G., S.B. (Lombard), S.M. (Illinois); Sc.D. (Harvard), Senior in Animal Breeding Investigation, Animal Husbandry Division, *Bureau of Animal Industry, Department of Agriculture, Washington, D. C.*

YERKES, ROBERT M., Ph.D. (Harvard), Professor of Psychology, University of Minnesota, Chairman Research Information Service, *National Research Council, 1701 Massachusetts Ave., Washington, D. C.*

YOCUM, H. B., A.B. (Oberlin), M.A., Ph.D. (California), Assistant Professor of Zoölogy, *University of Oregon, Eugene, Ore.*

YOSHIDA, SADAO, D.Sc. (Tokyo Imperial University), Professor of Parasitology and General Zoölogy in Osaka Medical College, Japan. *Home address, Osaka Medical College, Osaka, Japan.*

YOUNG, ROBERT T., B.S. (Pennsylvania), Ph.D. (Nebraska), Professor of Zoölogy, *University of North Dakota, University, North Dakota.*

ZELENY, CHARLES, Ph.D. (Chicago), Professor of Zoölogy, *University of Illinois, Urbana, Illinois.*

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, DECEMBER 6

TWO CASES OF MONOVENTRICULAR HEART WITH ATRESIA AND TRANSPOSITION OF SOME OF THE ROOTS OF THE GREAT VESSELS

JUAN C. NAÑAGAS

Department of Anatomy, University of the Philippines

EIGHT FIGURES (TWO PLATES)

In the course of a series of examinations that the writer undertook on a collection of Filipino infant hearts, for the special study of the foramen ovale, he encountered among 118 two instances of peculiar abnormalities that were thought of sufficient interest not only from the embryological viewpoint, but also from their physiological importance in relation to the viability of the fetus, as to warrant this brief descriptive report.

Congenital malformations of the heart do not usually interest many readers of medical literature, for the reason that they are often classed as "mere developmental curiosities of the human organism" devoid of special clinical significance to the general practitioner. Though this may be true to a certain extent in the two cases that I am now reporting, nevertheless, it must be borne in mind that some of these instances of defective cardiac development are quite compatible with life, and are of sufficiently common occurrence as to render their recognition not only important, but even essential to clinical medical men. Cases have been recorded where individuals have lived for years with cardiac malformations. In life, these patients must have undoubtedly exhibited definite but undiagnosed clinical pictures, which could have been recognized and a more rational treatment prescribed had their clinical consideration been carried in conjunction with corresponding anatomical and physiological studies. Gladstone, in his paper on cardiac malformations, emphasizes particularly this point when among other things he stated:

It is of greatest importance that the signs and symptoms produced by these different types of cardiac malformation should be carefully studied in all cases and recorded, so that an opportunity will be afforded of comparing one case with another, and of diagnosing the kind of abnormalities during life. In order to complete our knowledge of these cases, however, it remains for clinicians to correlate the signs and symptoms which these different malformations of the heart produce. It will then be possible to recognize the type of malformation during the patient's lifetime, and to carry out the treatment of individual patients upon a rational basis.

It is intended to present in this paper, first, the anatomical conditions of the abnormalities found in each case, and then to offer a tentative explanation of their possible embryological origin and the probable cardiac circulations occurring during intra and extra-uterine life.

ANATOMICAL FINDINGS

Case 1. The first specimen was the heart of a male Filipino infant that lived only two hours after birth. The case came to the city morgue from one of the health districts of the city, and for this reason no clinical history could be obtained except that the baby lived for about two hours. In opening the heart by routinary incisions I was surprised to observe that there were only three cardiac chambers, viz., the right and left auricles and a considerably enlarged ventricle. External examination showed that what should have been considered at a glance as the trunk of the pulmonary artery, with its prominent anterior position and left upward course, was really the ascending root of the aorta with its wide communication to the single ventricle. The real pulmonary artery was found only after a careful search, as a small poorly developed vessel on the left anterior side of the aortic trunk closely adherent to it, and apparently, from the outside at least, continuous with the base of the ventricle (fig. 1, *B* and *C*). The great cardiac vein together with the anterior coronary artery, that normally course downward to the apical notch of the heart, were considerably shifted to the right of the median line running obliquely to the right border of the ventricle (fig. 1, *A*).

This heart weighed 17.2 grams, or 0.81 per cent of the body weight, the latter being 2,114 grams. Its greatest circumference taken at the base of the ventricle was 96 mm. and its longest diameters were 46 mm. vertically, 37 mm. transversely, and 21 mm. anteroposteriorly. The ratio of the weight of the heart to the body weight in this case is a trifle greater than the normal ratio as given by Jackson (0.77 per cent) and Vierordt (0.76 per cent) in newly born infants. The difference of 0.04 to 0.05 per cent is in reality sufficiently small to warrant the conclusion that this heart, at least in weight, has attained its normal state of development.

On careful internal examination of the single ventricle no trace could be seen of the existence of any separation of this chamber into right and left halves. The right lateral wall, however, showed a marked thickening of the cardiac musculature, which extended medially, as far as a line coinciding externally, with the oblique course of the anterior coronary artery described above (figs. 2 and 3). An anteroposterior cut through the thickness of this part of the wall showed the muscle fibers in close relation anteriorly and superiorly with the rudimentary trunk of the pulmonary artery, from which point they appeared to stream obliquely downward and posteriorly. The excessive thickness of this part has produced a corresponding internal swelling into the cavity of the ventricle at its right lateral side. This fact would seem to indicate that this internal swelling probably represented the rudiment of an interventricular septum which might have become arrested in its development.

The single ventricle in this heart therefore is in reality the left ventricle which had become compensatingly enlarged. In the cavity of the ventricle there were found two large and several small papillary muscles and many trabeculae carnae (figs. 2 and 3). The principal papillary muscles were normal in location for the left ventricle, one of them arising from the left anterior wall and the other from the posterior wall. The cordae tendinae from these papillary muscles extended to the medial angles of the anterior and posterior cusps of the mitral valve and were attached to their margins.

The circumference of the left auriculoventricular orifice in this case was 35.5 mm. From cardiac measurements of ninety-six still-born infants made by me I had obtained a normal average of 24.5 mm. for the circumference of this opening. It seems, therefore, that the excess of 11 mm. would be accounted for by the fact that this orifice's being the only communication between the auricles and the ventricle it had probably become compensatingly enlarged.

The other communication that the single ventricle had was with the aorta. The circumference of this aortic orifice measured 16.5 mm., a little more than my normal average figure, which was 14 mm. This opening was guarded by three semilunar cusps, two anterior, slightly shifted to the left in the direction of the pulmonary artery, and one posterior. Each of these cusps showed well-developed sinuses, from two of which the right and left coronary arteries originated as in normal cases. The vessel leading from the ventricle through this orifice, though from its external position would correspond to the pulmonary artery, is nevertheless undoubtedly the aorta because of the facts above considered, viz., the position of the guarding semilunar valves and the origin of the coronary arteries and because of the greater thickness of its wall.

The right auriculoventricular orifice examined from the auricular side is oval in shape with its long axis directed transversely and its circumference measuring approximately 17 mm. Its margins are sharply defined and the flaps of the valve were completely fused together forming a 7-mm. pit underneath the orifice. Several small openings were found at the bottom of this pit which on a sagittal section showed them to open into blind crevices lodged in the right thickened part of the ventricular wall (figs. 2 and 3, *C*). This fact seems to further confirm the statement that the medial portion of this thickened wall represented a rudimentary interventricular septum. The crevices into which the openings of the flaps led were probably the remains of an imperfectly developed right ventricle, which conclusion is still more justified by the organic connection of the fibers of the ventricular wall in this neighborhood and the roof of the obliterated

pulmonary artery as previously recorded. The bundles of fibers found between these crevices were possibly diminutive papillary muscles.

The imperfectly developed trunk of the pulmonary artery was only about one-fifth the size of that of the aorta and was partly hidden from view by the left auricular appendix. On opening the artery proximally it was found to terminate blindly in three small sinuses which did not appear to have any connection with either the ventricle or the crevices found beneath the occluded right auriculoventricular orifice. Two of these sinuses were posterior and slightly medial in position, while the other was anterior. They were limited by three embryonic semilunar valves fused together and exhibiting thickened borders at the lines of fusion. Below this level the ventricular wall appeared on section to be entirely solid; the infundibulum or conus arteriosus was therefore entirely arrested in its formation. From the right posterior wall of the pulmonary artery about 2 cm. from its root arose the patent ductus arteriosus, which was about as large as the pulmonary trunk itself and established a free communication with the ascending aorta (fig. 1).

The foramen ovale was patent, the aperture between the free edges of the independent septum primum and septum secundum measured 4 sq. mm. The septum primum, however, was greatly relaxed, bulging considerably into the left atrium. This condition was possibly due to the irremediable free flow of blood from the right to the left sides of the heart.

Case 2. This heart was that of a poorly nourished full-term male Filipino baby that lived only four days postpartum. It showed harelip and polydactilism of both hands. Its body weight was 1,640 grams, sitting height 30 cm. and standing height 46 cm.

It will be noticed that the body weight in this case was far below the average given for normal newly born European and American babies as given by various investigators (over 3,000 grams) and a little below the average obtained by me for apparently normal Filipino still-born babies (2,200 grams).

Its standing height was only a trifle below the average figures given for American and European still-born babies, which was about 49 cm.

The differences of these two measurements from the normal averages would seem to indicate that while the development of the body was about normal, it was undoubtedly ill nourished.

From the shape of the heart alone one would readily anticipate the presence of some abnormalities. The vertical diameter was markedly in excess to the width. It weighed 17.7 grams, or 1.07 per cent of the body weight. This ratio was higher than in our first case and considerably greater than the figures given by Jackson and Vierordt. The greatest circumference of this heart was 92 mm., the largest vertical diameter was 47 mm., the transverse 36 mm., and the anteroposterior 26 mm.

The anterior surface represented by the anterior half of the right atrium and the ventricle was massive and much larger than the posterior surface. It was divided into a larger triangular right anterior facies and a narrower roughly quadrangular surface at the left by a linear elevation of the ventricular wall running from the base above to the apex of the ventricle below. The wall of the ventricle at the upper end of this right anterior portion was elevated in a marked upward prominence considerably deepening the auriculoventricular sulcus at this point (fig. 4).

The conus arteriosus was entirely absent and the pulmonary artery could not be seen on the normal point of its origin from the ventricles. The only vessel seen originating directly from the latter was the aorta, which appeared much larger than normal, and its ascending portion showed a funnel-shaped enlargement as it approached the arch. In position this vessel was shifted considerably to the left, arising from the upper third of the left ventricular border at a point considerably below the auriculoventricular sulcus. Unlike the ordinary condition, the aorta in this case followed an upward course, inclining immediately to the left so that its ascending portion was directly on the left upper ventricular wall, and only the arch came into relation with the left side of the atrium. The tip of the auricular appendix was barely touching the arch at its beginning.

On opening the right atrium by an incision following its greatest superior curvature, it was found to consist of an apparently dilated cavity into which three openings were found, two at its posterior wall corresponding to the superior and inferior venae cavae, and one found inferiorly leading into the ventricle, the auriculoventricular orifice.

On examining the posterior wall of this atrium from the outside, four smaller vessels, presumably the four pulmonary veins, were seen, which, however, did not directly open into the large cavity described above, but into a small flattened space adjoining the posterior atrial wall, which undoubtedly represented a rudimentary left atrium. This cleft-like space was separated from the enlarged right auricle by an interatrial septum in which an oval opening guarded by a thin septum was seen. This opening which was the foramen ovale appeared normal in every respect.

No communication between the left auricle and the ventricle was found. The only communication, therefore, between the ventricle and the atrial portion of this heart was represented by the right auriculoventricular orifice, which measured approximately 42 mm. in circumference.

Internally the ventricular portion of this heart showed only a single vertically elongated cavity, in which no recognizable division between a right and a left portion could be found. There was, however, a more or less prominent ridge situated in the middle of the posterior wall of the cavity, which by its position could be considered as representing the septum interventriculare (figs. 5 and 6). If such were the case, then this heart would have undivided right and left ventricles in their embryological condition, closely resembling in this manner the amphibian type of heart. The right portion of this ventricular cavity was in direct communication with the single enlarged atrium. The left side was continued upward and posteriorly to form the root of the aorta, while anterosuperiorly it gradually merged with a small elongated blind space found inside the peculiarly bulging portion of the anterior surface of the base of the ventricle (fig. 4).

The ventricular wall as a whole was massive, averaging about 6 mm. in thickness. Two small papillary muscles were present,

one situated in front and the other posteriorly, with their cordae tendinae attached to the edges of the flaps of the single auriculoventricular orifice. This opening possessed but two flaps, differing in this respect from the normal, which has three.

The aortic opening, as in normal hearts, was guarded by three semilunar cusps, two found anteriorly and one posteriorly. The three aortic sinuses (sinuses of Valsalva), were found to be dipping down into the wall of the ventricle to a much greater extent than in normal cases. The ascending aorta, as noted above, was funnel-shaped and joined the aortic arch upward posteriorly. The latter was likewise fairly dilated.

Only the right coronary artery originated from the right aortic sinus. It coursed downward in a zigzag direction for a short distance in the posterior coronary sulcus, which it soon left to run along the posterior or diaphragmatic surface of the heart, curving toward the left border considerably above the apex, and anastomosing at this point in a plexiform manner with the anterior descending branch of the left vessel. The left coronary arose directly from the anterior wall of the ascending aorta. On reaching the level of the root of this vessel in its descent, it followed forward and to the right, the anterior coronary sulcus under cover of the right auricle until it reached its extreme right end; thence the artery coursed along the right ventricular margin as far as the apex, where it resolved into plexiform anastomoses.

Another striking anomaly found in connection with the aorta was the presence of two fairly good-sized branches originating from its posterior wall about half a centimeter from its proximal end. These vessels were found to correspond with the right and left pulmonary arteries, the common pulmonary trunk being absent. This peculiar condition was not noticed until after the removal of the heart, and the identification of these two vessels was based upon a careful search and topographic comparison of their cut cardiac and pulmonary ends. The diameters of these two vessels were approximately 4 mm. for the right and 3 mm. for the left.

REVIEW OF LITERATURE

In reviewing the literature at our command for cases showing similar cardiac malformations, we found the following:

1. Three-chambered hearts. Keith ('09), in his extensive review of 272 cases, established three types of three-chambered hearts, viz., *a*) hearts in which the interventricular septum was so little developed, or in which the interventricular foramen was so large that the two ventricles were rightly said to form one chamber; *b*) those cases where the right ventricle was suppressed by the septum's being applied to the right side of the heart, and, *c*) those in which the left ventricle was suppressed by the interventricular septum closely shifted to the left ventricle wall of the ventricle.

He found in his set nine cases belonging to the first type with associated arterial transpositions; seven cases to the second type, five of which showed complete obliteration of the right ventricle and two with the two ventricles forming a common chamber, and in every case the infundibulum was well developed. Of the third type he found five cases. In the second and third groups the absence of a ventricle was associated with a complete or almost complete obliteration of the corresponding auriculo-ventricular orifice.

Young ('07) and Dixon Mann ('07) reported a case of an adult man aged 35 years whose heart showed complete absence of the septum interventriculare with associated transposition of the arterial trunks. Paterson ('08–'09) reported a similar cardiac malformation in a man 22 years old. Both cases belong properly to the second type.

Gladstone ('15–'16) cited two cases of this cardiac anomaly, one from a child 48 hours old where the left ventricle was rudimentary and functionless and which therefore would fall under group 3, and the other case, a woman 50 years of age with a heart showing a patent foramen interventriculare with constriction of the ostium bulbi; which could be included under type 1.

Dickson and Frazer ('13) had a case in a male child 3 months old. The specimen showed a hypertrophy of both ventricles

with incomplete development of the interventricular septum. This instance could be included in the first type. It was asso ciated with an abnormal origin of the pulmonary artery.

Keith ('12) mentioned four other cases where the interventricular foramen showed varying degrees of patency found in individuals from 9 months to 16 years of age, associated with such anomalies as closure of the auriculoventricular orifice, undeveloped ventricle, and closure of the pulmonary orifice, etc. Three of these cases properly belonged to the first type, but one case, with undeveloped condition of the right ventricle, could also be considered as belonging to type 2. He also cited a case of a child 48 hours old where the heart showed obliteration of the left ventricle and the corresponding auriculoventricular orifice. This case of course belonged to type 3.

Black ('13–14) reported two cases: one heart, belonging to an adult male 22 years of age, showed a large interventricular foramen with associated malformations of the pulmonary cusps; the other was that of a male child 6 months old with a patent foramen interventriculare and a marked reduction of the size of the aorta with no associated anomaly of the infundibulum. These two might rightly be included under type 1.

Our first case belongs to type 2, as it shows a diminutive right ventricle with the interventricular septum shifted to the right side. The second case that had an incomplete septum is to be included in type 1 of Keith's classification. In case 1 there was a complete obliteration of the right auriculoventricular orifice from fusion of the flaps. Case 2 showed a complete absence of the mitral opening.

2. Arrest in the developmental expansion of the infundibulum. Keith ('09) encountered thirty-seven cases of such condition. The typical picture of such cases had the infundibulum reduced to a mere slit situated at the orifice of the pulmonary artery which was generally represented by a small cicatricial mass, or a very small lumen in which the fused semilunar valves could be distinguished. The pulmonary artery was in the majority of cases represented by a fibrous cord at its origin.

In the case of Dickson and Fraser ('13) the right pulmonary artery originated from a point $\frac{1}{2}$ inch above the commencement of the ascending aorta and the left from the descending portion.

Keith ('12) mentioned a case in a male child 11 months old where the infundibulum was obliterated and the pulmonary artery received blood from the ductus arteriosus.

Peterson's ('08–'09) case showed besides, a marked stenosis of the pulmonary orifice with a substitution of the pulmonary valves by a thick pad of cardiac tissue.

In our cases, the first showed the pulmonary arterial trunk markedly reduced in size and the pulmonary orifice entirely closed from fusion of the semilunar flaps. In the second case the pulmonary trunk was entirely absent, and the two small arterial vessels identified as the pulmonary arteries were seen arising from the posterior side of the aorta.

3. Atrophy of the auricular portion of the heart. Keith ('09) met three cases which showed considerable diminution in the size of the left auricle associated with complete stenosis of the mitral orifice. He stated that such cases might easily have escaped detection and could have been considered as two-chambered hearts—a rare condition which, even with his wide experience on the subject, he had never yet seen.

Our second case shows undeveloped left atrium together with absence of the left auriculoventricular orifice.

4. Incomplete separation of the aorta and pulmonary artery. Irregularities in the division of the truncus arteriosus, which becomes divided to form the root of the aorta and the pulmonary artery, according to Keith, are rare. He had only seen three cases in his series. Rakitansky reported a case with this condition, but with the pulmonary artery considerably larger than that found in our case.

Our second case showed incomplete separation of the pulmonary artery from the ascending aorta and two small vessels representing the pulmonary arteries arising directly from the posterior side of the beginning of the aortic root, which in turn was considerably shifted to the left side of the heart.

EMBRYOLOGICAL CONSIDERATIONS

Owing to the various possibilities which could be considered in our cases, it would seem rather difficult to definitely offer any explanation that would adequately cover the different embryological factors which might have led to the production of the anomalies herein enumerated.

1. Monoventricular condition. In our first case we encountered a suppression of the right ventricle by the septum's being applied to the right side of the heart. In the second case the two ventricles were imperfectly separated by defects in the interventricular septum.

We have failed to find in our search of the literature any author who endeavored to explain the first condition.

It is a well-known fact that at some stage of the normal development of the heart there is a time when the so-called ventricular limb starts its division into a greater left and a smaller right half, to be later known as the left and right ventricles, respectively, by the production on its external convex surface of a groove and within its cavity of an interventricular septum.

If at this period of development by some unknown reason the normal proportionate development of parts were arrested or at least changed to an unequal growth, similar anomalies would probably result. Thus if we were to suppose at this stage a delayed or even an arrested growth of the right side of the heart, with consequent continued and relatively increased development of the left, a condition would result where the left cardiac cavity would appear greatly enlarged, with the septum apparently reduced to a mere parietal thickening shifted well to the right, and the right ventricular cavity represented by mere crevices or by a diminutive cavity hardly large enough to permit the unfolding of the tricuspid valves. This condition is exactly similar to that encountered in our first specimen.

Our second case of monoventricular heart with imperfect separation of the two ventricles by defect in the interventricular septum has been encountered and explained by several writers on this subject. Gladstone explains the persistence of the inter-

ventricular foramen as an apparently secondary condition to the failure in the expansion of the bulbar portion of the right ventricle, and says that its presence and size depend largely upon the extent of the defect. Hunter and Keith hold the same view, i.e., that the interventricular foramen is produced by the obstruction or stenosis of the pulmonary artery and that the closure of the interventricular foramen depends on the complete development of the infundibulum. Keith further states that from 85 to 90 per cent of the cases with pulmonary stenosis showed an interventricular foramen.

Meckel, as cited by Keith ('09), claimed, on the other hand, that the interventricular foramen is a primary condition and the pulmonary stenosis its sequence.

Young asserts that the course of the main blood-stream inside of the heart could interfere with the complete development of the interventricular septum.

The monoventricular condition in our second case may be explained perhaps by an arrest of growth of the interventricular septum, which in this case is represented by a mere ridge in the ventricular floor. This septal condition is similar to its early stage of development in the division of the embryonic ventricle into two halves. This arrest is undoubtedly due to the mechanical pressure and friction exerted upon it by the flow of blood coming through the right auriculoventricular orifice and which not finding any exit at the right ventricular side because of the total absence of a pulmonary artery is forced toward the left side of the ventricle to find there its only orifice of escape represented by the aorta (fig. 8).

The abnormal and excessive upward prominence of the ventricular wall in its anterosuperior base must also be looked upon as a necessary result of the same continued increased pressure exerted upon that part of the ventricle by the blood-stream passing through the aortic orifice. This influence is certainly conceivable, if we remember that the arterial pulmonary trunk should have taken origin from this region, and that the tender embryonic cardiac musculature is very apt to give way to such a persistent and continued mechanical factor.

2. Congenital stenosis of the pulmonary orifice with or without arrest of the developmental expansion of the infundibulum. Keith explains the majority of those cases of congenital pulmonary stenosis associated with arrested development of the infundibulum as due to a failure of the bulbus cordis to incorporate with the right ventricle. In those cases where the stenosis is not, however, associated with an arrested infundibulum, he believes foetal endocarditis to be responsible for the defect.

In this connection we quote from Gladstone the following:

> According to Schipman, the most common and important effect of intra-uterine endocarditis is stenosis of the pulmonary orifice, and he also states that the limitation of the endocarditis to the right side of the organ during intra-uterine life comes under the same law as its limitation to the left side in extra-uterine life. In both cases it is the cavity which has most work to perform that is affected. According to this view, cases in which the interventricular foramen has not persisted are produced by endocarditis occurring after the period when this foramen is normally closed. The closure is effected by the fusion of the interventricular septum with the bulbar septum, and this takes place in embryos between 12 mm. and 18 mm. in length, and at the age of six or seven weeks. It is possible, however, that the endocarditis is a secondary incident which has occurred after birth, and that the primary condition is constriction. It is probable also that the constriction is attributable to a primary defect in development rather than to a foetal endocarditis; for if foetal endocarditis were the cause, we should expect also to find stenosis of the right auriculo-ventricular orifice with endocarditis of the tricuspid valve, but this is not found, on the contrary congenital stenosis of the right auriculo-ventricular orifice is extremely rare.

In our two cases we found a complete absence of the infundibulum, associated in the first case with a diminutive pulmonary artery and stenosis of the pulmonary orifice by fusion of its similunar valves and with complete absence of the pulmonary artery and pulmonary orifice in the second.

These defects plus the absence of any inflammatory signs in the perfectly smooth and shiny fused pulmonary flaps incline us to believe that neither foetal endocarditis nor the failure of incorporation of the bulb into the right ventricle would furnish satisfactory explanations.

In normal cases we know that the separation of the bulb into the aortic and pulmonary trunks is the result of the fusion of the right and left distal bulbar swellings and the septum aortopulmonale. These swellings are linear thickenings of the endocardial surface of the bulb, two at its proximal and four at the distal portion. The union of the right and left distal bulbar swellings is generally produced by the ingrowth of the connective tissue of the bulbar wall. This fusion together with the approaching septum aortopulmonale brings about the complete division of the bulbar trunk into a ventral vessel, the aorta and a dorsal pulmonary artery. The two proximal swellings by their spiral direction complete further this division by bringing each vessel into a more direct relation with its corresponding ventricle. The semilunar valves of the aortic trunk are formed by the anterior distal bulbar swelling and the two anterior halves of the right and left laterally. Those of the pulmonary artery by the posterior and the posterior halves of the right and left distal lateral swellings.

Now it is possible that in the first case, owing to inequality in growth of the ventricle, the union of the right and left bulbar swellings with the septum aortopulmonale has taken place, but considerably shifted to the anterior side of the bulb. This shifting has necessarily reduced the pulmonary artery to a diminutive trunk, and its semilunar valves, remaining diminutive and crowded together, have become completely fused.

In the second case the septum aortopulmonale has probably failed to descend and meet the bulbar swellings, leaving in this manner the truncus arteriosus undivided, and the pulmonary arteries arising directly from the ascending aorta. Such condition would also account for the absence of the pulmonary orifice.

3. Rudimentary and collapsed condition of the left auricle. As regards the rudimentary and collapsed state of the left atrium and the absence of its atrioventricular orifice, it does not seem possible to offer as reasonable explanations as in the anomalies already considered above, we might, however, conceive such abnormality as due to a probable combination of such two factors as:

a. A possible arrest or entire failure of development of the two endocardial cushions which should normally appear in the ventral and dorsal walls of the heart in the region of the atrioventricular sulcus, together with fusion of this sulcus with the interatrial septum in the side of the left atrium.

b. Normally developed cushions which after their union were shifted to the left to meet and fuse with the atrioventricular sulcus in this side. This condition, if combined with an arrest of further development of the left atrium with a compensatory enlargement of the right side, would give rise to a condition similar to that found in our specimen.

Such a combination of factors influencing the production of the above abnormalities could only be made possible by the total absence or diminished amount of blood flowing into the left atrium as a result of the absence of the arterial pulmonary trunk.

The four pulmonary veins probably transmitted little blood if any at all into the left atrium, two vessels which were taken as representing the pulmonary arteries being altogether too small to carry on any appreciable pulmonary circulation.

PHYSIOLOGICAL CONSIDERATIONS

Probable cardiac circulation in the two cases. A morphological study of the circulation, both during the intra-uterine and postpartum lives, in both cases would indeed be of some interest.

Case 1. Prenatal circulation in this case is obviously possible and could normally be carried on to full term without greatly impairing the normal general development of the foetus.

The arterialized blood from the placenta undoubtedly followed a normal circuit by passing successively through the right and left atria (freely communicating with each other by a patent and relaxed foramen ovale), to the single ventricle through the widely open mitral orifice, and thence to the general circulation, passing out of the aortic root.

A satisfactory explanation of the postpartum circulation in this case would offer several difficulties. Literatures on cardiac malformations mention only a few instances where three-cham-

bered hearts were found in adult individuals. Cases with complete atresia of the pulmonary orifice, as in the case under consideration, have never been known to live for more than twenty-four hours. In such instances where a complete arrest of the infundibulum was present, the lungs, according to Professor Keith, were supplied with blood from the systemic circulation through the bronchial arteries and other accessory branches of the intercostals. It is unfortunate that we were unable to obtain a complete clinical history of our case; this would probably have helped us in our explanation by knowing the behavior of the child after delivery.

It is of course possible to conceive that the pulmonary circulation could be carried in one of the two following ways:

a. The lungs may receive some blood via the bronchial arteries and infercostal branches, as explained by Professor Keith. We regret that we were unable to study the bronchial arteries of this specimen. We question, however, the ability of such circulation to sustain life for any length of time in our case because of the almost complete absence of any expanded lung tissue in either side. The child lived only two hours.

b. Pulmonary circulation might be possible also by the blood passing successively from the right auricle to the left atrium through the foramen ovale, thence to the left ventricle through the mitral orifice, therefrom into the aorta from which a small amount of blood could enter the ductus arteriosus in the reverse direction and eventually reach the lungs via the pulmonary artery (figs. A and B).

The great downward obliquity of the ductus arteriosus in its union with the aorta, its relatively small size, and the reverse direction of the collateral stream incline us to believe that only a small amount of blood, if any at all, could possibly reach the lungs by this route. If we add further to the above facts, the venous condition of the blood thus circulating, we almost wonder how the child could have lived even for two hours as it did, unless the oxygenation of its blood from the placenta could have carried him through that length of time.

Case 2. The foetal circulation in utero in this case undoubtedly followed a similar route as in case 1.

The postpartum circulation offers to a certain extent similar difficulties as those encountered in case 1.

The pulmonary circulation was carried on again either by the collateral bronchopulmonary circuit of Professor Keith or through the two diminutive pulmonary arteries branching off from the aorta. In either case, however, the return circulation took place through the four diminutive pulmonary veins into the collapsed left atrium and thence through the foramen ovale into the right auricle, completing in this manner the pulmonary circuit.

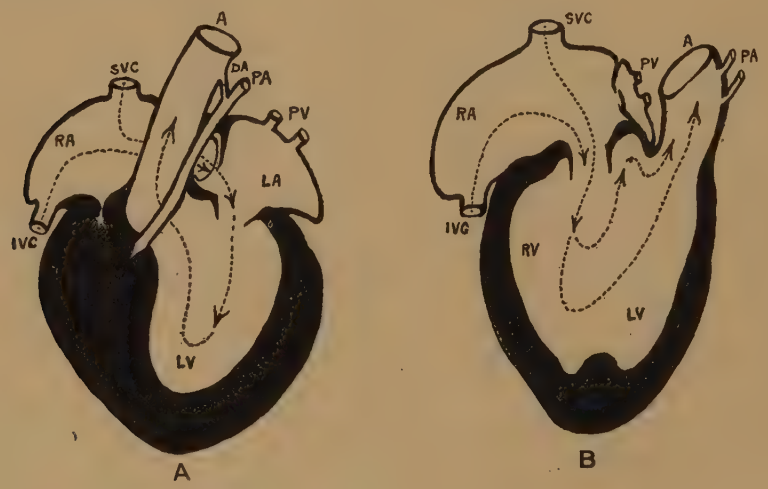


Fig. A Diagrammatic sketch showing probable circulation in case 1.
Fig. B Diagrammatic sketch showing probable circulation in case 2.
IVC, inferior vena cava; *RA*, right atrium; *LA*, left atrium, *SVC*, superior vena cava; *A*, aorta; *DA*, ductus arteriosus; *PA*, pulmonary arteries; *PV*, pulmonary veins; *LV*, left ventricle; *RV*, right ventricle.

We believe, however, that the amount of blood reaching the lungs by any one of the above two routes, though probably was greater in amount than in the first case, yet it was without doubt insufficient to support life for any long period. We base this opinion on the great reduction of size of the pulmonary arteries and veins (less than one-third the normal dimensions) and the diminutive collapsed condition of the left atrium, both

of which spell a proportionately reduced blood stream in the entire circuit, which though sufficient to enable the foetus to live for a few days, is nevertheless undoubtedly incompatible with prolonged life. This case lived about four days.

CONCLUSIONS

In closing, we would like to draw attention to the following points of interest:

1. The incidence of monoventricular hearts among the Filipino babies is apparently not a very rare condition. In our series we encountered them in the proportion of 1.6 per cent. This fact would seem to call for a more careful clinical study of newly born babies showing obscure cardiac symptoms with fatal results.

2. That the ratio of the weight of the heart to the body weight in our cases was considerably greater than the normal ratio as given by Jackson and Vierordt, denoting undoubtedly a compensatory effort on the part of the heart to carry on circulation under abnormal conditions.

3. That not all cases of persistent interventricular foramen can be explained by the failure of expansion of the bulbar portion of the right ventricle or by pulmonary stenosis produced by foetal endocarditis. In many instances it is necessary to invoke also defects of growth, mechanical effect of abnormal blood stream, etc.

4. That the congenital atresia and stenosis of the pulmonary orifice in our cases was probably due to either irregularity of growth of the ventricle and shifting to one side of the union of the right and left bulbar swellings and the septum aortopulmonale or to the entire failure of the septum aortopulmonale to unite with the distal lateral bulbar swellings.

5. That monoventricular hearts are perfectly compatible with normal development in intra-uterine life, but when associated with pulmonary stenosis, even with patency of the ductus arteriosus, it is probable that life cannot continue for more than a few days, although the collateral pulmonary circulation might be taken up by branches of the bronchial and intercostal arteries.

I wish to acknowledge my indebtedness to Dr. H. W. Wade, of the Department of Pathology, for allowing me to report case 2, which came to them at the autopsy table, and to Dr. Arturo Garcia for some helpful suggestions in the preparation of this paper.

LITERATURE CITED

Black, Davidson 1913–14 Two cases of cardiac malformation—more especially of the infundibular region. Journal of Anatomy and Physiology, vol. 48, p. 274.

Dickson and Fraser 1913 A congenital abnormality of the heart and blood-vessels. Journal of Anatomy and Physiology, vol. 48, p. 210.

Gladstone, R. J. 1915–16 Two examples of cardiac malformation. Journal of Anatomy and Physiology, vol. 50, p. 228.

Keith, Arthur 1909 Malformations of the heart. Lancet, vol. 2, pp. 359, 433, and 519.

1912 Six specimens of abnormal heart. Journal of Anatomy and Physiology, vol. 41, p. 190.

Mann, J. Dixon 1907 Cor Triloculare Biatriatum. Brit. Medical Journal, vol. 1, March 16.

Paterson 1908–9 Congenital malformed heart. Jour. of Anatomy and Physiology, vol. 43, p. lv.

Young, A. H. 1907 Rare anomaly of the human heart—A three-chambered heart in an adult aged thirty-five years. Journal of Anatomy and Physiology, vol. 41, p. 190.

PLATES

PLATE 1

EXPLANATION OF FIGURES

1 Anterior view of heart of Case 1. A. Anterior coronary artery and great cardiac vein. B. Aorta. C. Pulmonary artery.

2 Anterior half of coronal section of above heart (case 1). A. Aortic orifice. B. Right auricle. C. Crevices representing remains of right ventricle. V. Ventricle (single).

3 Posterior half of coronal section of above heart (case 1). B. Right auricle. C. Crevices representing remains of right ventricle. D. Ventricle (single). E. Left auricle.

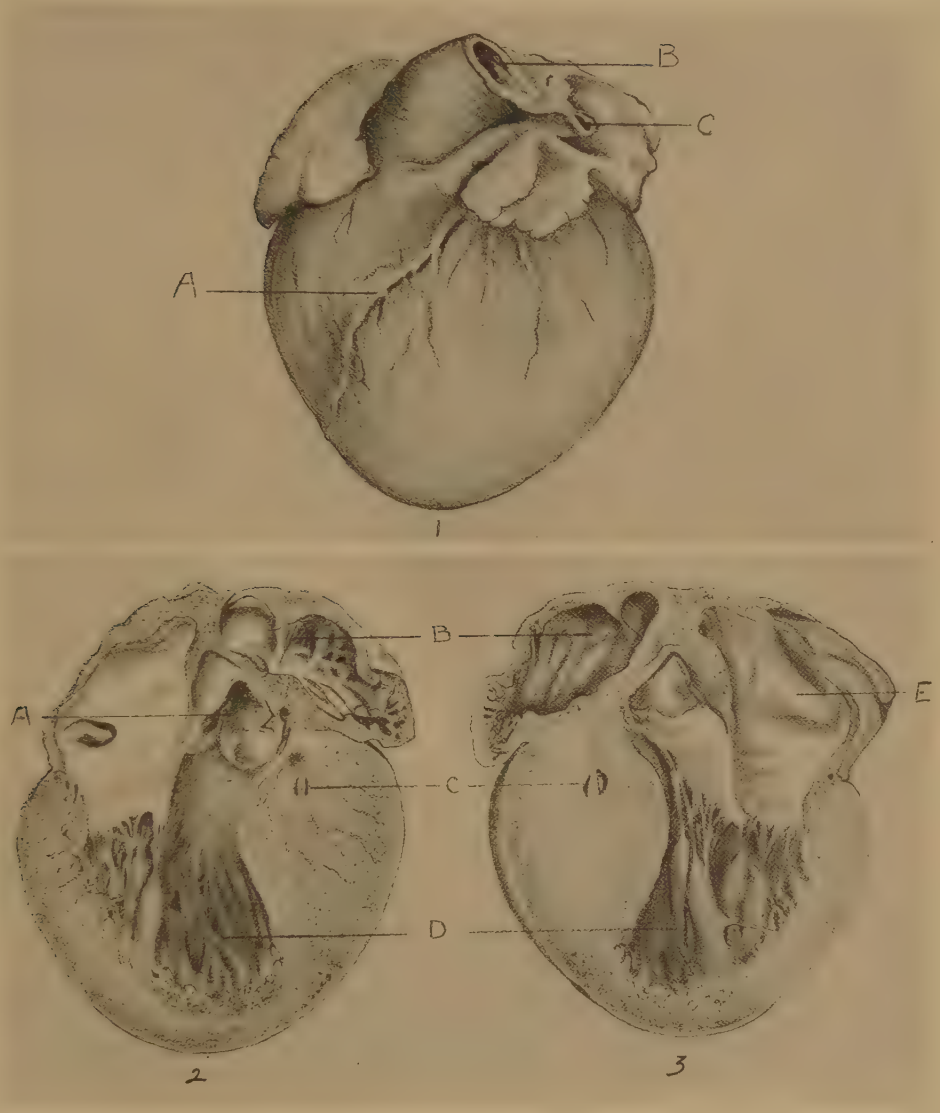

B
C
A
1
B
A
C
D
E
2
3

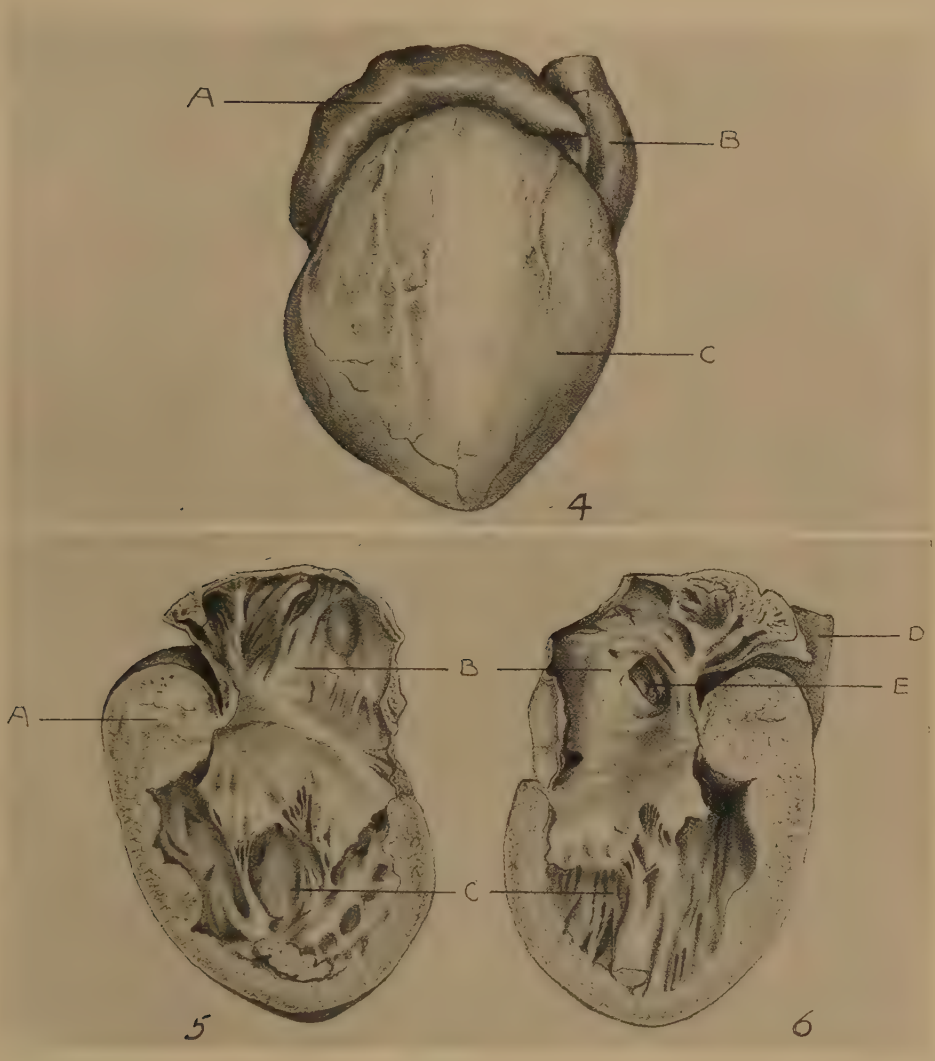
PLATE 2

EXPLANATION OF FIGURES

4 Anterior view of heart of case 2. A. Right auricle. B. Aorta. C. Ventricle.

5 Anterior half of coronal section of above heart (case 2). A. Thickened upper part of left wall of single ventricle. B. Right atrium. C. Single ventricle.

6 Posterior half of coronal section of above heart (case 2). B. Thickened upper part of left wall of single ventricle. C. Single ventricle. D. Descending aorta. E. Foramen ovale.


A
B
C
4
A
B
C
5
D
E
6

Resumen por el autor, Harvey Ernest Jordan,
Universidad Washington, Saint Louis.

Nuevas pruebas sobre la función de los osteoclastos.

El autor presenta pruebas histológicas sobre la función histolítica de las células gigantes multinucleadas de la médula ósea mandibular del gato recién nacido. Estas células gigantes contienen glóbulos voluminosos de material óseo reabsorbido, y son osteoclastos genuinos. La pulpa del órgano productor del esmalte contiene también células gigantes semejantes, provistas de glóbulos similares. Estos últimos glóbulos son considerados por el autor como esmalte superfluo reabsorbido y las células gigantes como ameloblastos.

Translation by José F. Nonidez
Cornell Medical College, New York

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, DECEMBER 27

FURTHER EVIDENCE CONCERNING THE FUNCTION OF OSTEOCLASTS

H. E. JORDAN

Department of Histology and Embryology, University of Virginia Medical School

FIVE FIGURES

In a recent paper on the giant cells of the yolk-sac and the bone marrow, the writer (*4*) included a brief description of 'osteolytic giant-cells' ('osteoclasts' of Koelliker) in the mandibular marrow of the new-born cat. In this paper a distinction was made between the 'hemogenic' and the 'osteolytic' giant-cells of the bone-marrow. The hemogenic giant-cells were shown to develop by excessive growth from hemoblasts. By amitotic division of their transiently polymorphous nucleus these cells become multinucleated, and in this condition occasionally differentiate intracellular erythrocytes. The osteolytic giant-cells 'originate by the aggregation of many nuclei in certain areas which then become separated to form large irregular syncytia, which may fuse with or incorporate osteoblasts and bone cells. These syncytia become placed upon spicules of bone, which they resorb' (p. 241) (*4*). The hemogenic giant-cells are accordingly comparable to multiple erythroblasts or blood islands; the osteolytic giant-cells are comparable to foreign-body giant-cells. In the osteoclasts were described globules of a substance with the identical structure and staining reaction of the adjacent spicules of bone. These globules were interpreted as resorbed osseous material. This observation constitutes the sole recorded direct evidence that the so-called osteoclasts actually ingest bony substance. The only other approximately direct evidence that these giant-cells possess an osteolytic function is that recorded by Koelliker (*5*). Following Tomes and de Morgan (*7*), and Billroth (*2*) he drove ivory pegs into bone. Subsequent study

of this experimentally modified material showed an extensive pitting of these pegs, with multinucleated giant-cells occupying an occasional pit. Tomes and de Morgan, and Billroth, however, regarded these pits in the ivory pegs as eroded through the agency of connective tissue.

Arey (*1*) questions the adequacy of the evidence above outlined as signifying a specific osteolytic function on the part of the so-called osteoclasts. He concludes, moreover, that the hypothesis 'that they are merely degenerating, fused osteoblasts, accords better with the known facts' (p. 335). It seemed desirable, therefore, to devote a renewed and more extensive study to my preparations in which the giant-cells showed these 'osseous globules.' The chief purpose of this investigation is to enlarge the body of evidence touching the function of these giant-cells as judged by their cytology and to subject the same to a more rigidly critical analysis. Questions concerning the origin and fate of the osteoclasts will not be here further considered. It is necessary to draw a sharp distinction in this connection between an osteolytic or osteoclastic function and a mere phagocytosis of already fragmented and decalcified bone of resorption foci. The evidence seems unequivocal as concerns an osseophagic function of these cells. The central point at issue concerns the active and efficient agent in the dissolution and decalcification of the disappearing spicules of bone in bone development and growth. It should be stated here that Koelliker (*6*), in his second extensive paper on bone resorption, no longer regards his experimental evidence with ivory pegs as conclusively indicating a specific osteolytic function of the osteoclasts. He inclines to ascribe some osteolytic function in growing bones also to the medullary and periosteal connective tissue.

The material upon which this investigation is based consists of sections of the lower jaw of a new-born cat. The tissue was fixed with Zenker's fluid, decalcified in a 5 per cent aqueous nitric-acid solution, imbedded in celloidin, cut at 12μ, and stained with hematoxylin and eosin. We are interested here in both the alveolar bone and the included teeth; that is, in the multinucleated giant-cells with osseous globules both of the bone-marrow and of the enamel pulp.

The character of the material is shown in figure 1. The bone is of the cancellous membranous type, with remnants of 'cartilage bone' formed in Meckel's cartilage of the fetal mandible. The tooth is of course of the deciduous series, and of a stage of development several months prior to eruption. The tooth is

Fig. 1 Photomicrograph of transverse section of lower jaw of new-born cat, including tooth. × 12. Over the crown of the tooth the remnant of the enamel pulp has shrunken away from the enamel. Photos by Mr. William S. Dunn, Cornell University Medical College, New York City.

enveloped apically by the primordium of the future cuticular membrane (of Nasmyth) consisting of remnants of the ameloblasts, the enamel pulp (with its inner stratum intermedium), and the peripheral enamel epithelium. The enamel pulp contains numerous multinucleated giant-cells, some with globules of a material apparently identical with that of the osteoclasts, others in process of degeneration and disintegration.

We may to best advantage consider the osteoclasts first. As already stated, these contain larger and smaller globules of a substance with apparently the identical homogeneous structure and acidophilic staining reaction as the spicules of membrane bone (figs. 3 to 5). These ingested globules are commonly located in the portion of the giant-cell next the bone; but occasionally they may apparently lie in the portion farthest removed from the bone (figs. 4 and 5). The latter condition in certain cases is to be interpreted in terms of an adjacent spicule of bone in a higher or lower plane. In figure 4 is shown a giant-cell in process of ingesting a large pestle-shaped mass of free bone. These illustrations would seem to admit of no doubt concerning the osseophagic capacity of these giant-cells. The globular, sharply contoured form of the phagocytosed osseous material indicates that it is ingested only in a fluid or semisolid condition; that is, after a preparatory decalcification of portions of the bony spicule. In figure 5, at the left, is shown a large, relatively clear, circular area, probably representing an osseous globule in a late stage of digestion.

The suggestion has been made to me that these globules of bone which I interpret as ingested free portions of bone are actually only extensions of bone within the giant-cells from adjacent spicules. Such suggestion has no pertinency to my descriptions of intracellular globules since it can be proved, by raising and lowering the level of focus, that many globules are actually within the giant-cell protoplasm. An interpretation that presented itself very forcibly to me, not hitherto suggested, and one which I have now thoroughly tested, is that these alleged osseous globules are actually only ingested red blood-corpuscles which either remained isolated or became fused into homogeneous masses. It cannot be denied that these giant-cells do phagocytose erythroplastids as well as osteoblasts and bone cells. Also, some of these smaller globules have approximately the size of a spheroidal erythroplastid. But the red blood-corpuscles of these specimens have a different shape and staining reaction, both in the adjacent blood-vessels and after ingestion by giant-cells. They occur either as cup-shaped,

crenated, or circular biconcave discoid bodies; or they may be variously irregular, or even fragmented. Furthermore, they never exhibit the same deep bluish-red staining reaction; the red blood-corpuscles are either brownish yellow, pinkish brown or light pink in color. In a section of human femoral marrow from a case of fatal typhoid fever, treated with a similar technic, many mononucleated cells can be seen engorged with red blood-corpuscles in all stages of disintegration. In no case, however, is the ingested group of corpuscles fused into a homogeneous mass similar to the globules here described in the osteolytic giant-cells. Moreover, I can find no mention of any condition, either in normal or pathologic histology, where erythroplastids are fused into comparable globules. Nor can these globules be regarded as hyaline degeneration products in view of the generally healthy appearance of these giant-cells.

The question arises as to the active agent in the decalcification and the fragmentation (resorption) of the bone, making possible the ingestion by these giant-cells. There are three obvious possibilities: 1) the medullary connective tissue; 2) the blood-vessels; 3) the giant-cells. If the connective tissue were the specific osteolytic agent in this tissue, we would expect to find numerous globules of bone scattered throughout at least that portion adjacent to the bone spicules. But such globules are lacking in the bone-marrow except occasionally in close proximity to the giant-cells. If the blood-vessels were the effective causative agent in osteolysis in this growing bone, we would expect to find the globules to some extent within the vessels or at least in close proximity to them. Such conditions also do not obtain. Moreover, the vascular supply seems too meager, and is is not generally sufficiently close to the spicule of bone, to furnish plausible support to this assumption. By process of elimination of obviously possible osteolytic agents there remains the osteoclast. This certainly contains globules of ingested osseous material. It is invariably present where bone is being resorbed and reshaped at least during early stages of development. It is generally in close connection at one or several points with the peripheral layer of the bony spicule (figs. 4 and 5).

The evidence from the bone-marrow above described would seem to leave no escape from the conclusion that the giant-cells in this tissue are the active proximate agents in the decalcification and fragmentation, and subsequent ingestion (resorption), of the bone; that is, that they are genuine osteolytic giant-cells or osteoclasts, as originally claimed by Koelliker (*5*). But this conclusion must be further appraised in the light of data accruing from the study, next to be reported, of the multinucleated giant-cells of the enamel pulp.

I was at first very much surprised to find what appeared to be identical globules both in the general syncytium and in certain multinucleated giant cells of the enamel pulp of the deciduous pre-erupted teeth in these preparations (fig. 2). Globular shape, variable size, sharp contour of borders, homogeneous structure, and similar staining reaction force the conclusion that these globules of the enamel pulp are practically identical with those above described for the giant-cells of the bone-marrow. In the case of the enamel organ, with its 'pulp,' we are dealing with an ectodermal structure invaginated into the primitive jaw from the overlying epithelium. The cells of the basal layer differentiate into ameloblasts which function in the production of the enamel; the intermediate layers form a loose syncytium, and the peripheral layer becomes a thin membrane with cells of endothelioid form. The erupting tooth remains covered with an atrophic membrane, the cuticular membrane of Nasmyth, composed of the nucleated remnants of the ameloblasts, the compressed tooth pulp, and the greatly flattened peripheral enamel epithelium. The erupted crown may remain covered with only ameloblastic remnants.

The possibility presents itself that the 'osseous globules' of the enamel pulp are transported from the marrow, or vice versa. If this were the correct interpretation, such globules should be found in the portion intermediate between the crown of the tooth and the deeper bone-marrow; that is, in the paradental corium of the jaw. The agents in such transference would conceivably have to be either blood-vessels or amelopulpar giant-cells; or possibly the globules might find their way from the

enamel pulp to the alveolar areolae through the operation of gravity. The last suggestion may be dismissed as quite improbable, more especially in view of the fact that no free globules of this sort are found in the intervening connective tissue. If

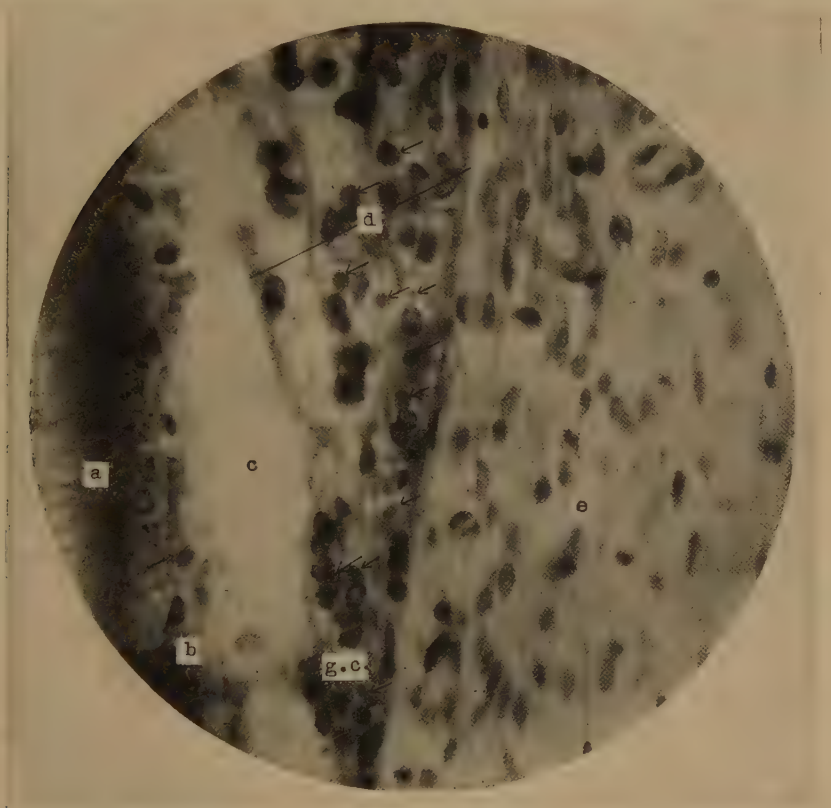


Fig. 2 Photomicrograph of area X of fig. 1. × ca. 600. *a*, enamel; *b*, stratum intermedium of enamel pulp; *c*, fixation (contraction) artifact; *d*, enamel pulp; *e*, connective tissue of the dental sac; *g.c.*, two small multinucleated giant-cells, containing enamel globules. The arrows point to some of the globules within the pulp syncytium. These globules do not differentiate themselves sharply in the photograph from the nuclei of the pulpar cells.

these globules passed either way via the blood-vessels, we would expect to find them also within these vessels; but such globules are entirely absent from the blood-vessels. Moreover, the enamel organ is non-vascular. The terminal blood channels

stop short abruptly upon the outer enamel epithelium of the enamel organ. As regards the giant-cells, none are found just outside the enamel organ in the connective tissue intervening between the lateral border of the tooth and the underlying bone. The possibility of a transportation of these globules from the enamel pulp to the marrow areolae of the alveolar bone or vice versa seems definitely untenable.

We are then confronted with the demand for an explanation of the occurrence of these 'osseous globules' within the enamel pulp and the included giant-cells. These giant-cells are formed in a manner quite similar to that by which the giant-cells of the alveolar bone arise, that is by closer fusion of 'cells' of the pulp syncytium. They form, therefore, in the manner of foreign-body giant-cells. These giant-cells of the enamel pulp certainly ingest the osseous globules; many of which lie free within the pulp, or in vacuoles in the pulp cells, and especially as large free, spheroidal and greatly elongated, globules next the enamel. The giant-cells are phagocytic for these globules, and they subsequently disintegrate by a process initiated by a general karyorrhexis of the multiple nuclei, a stage of which gives a simulacrum of multipolar mitoses. During this process of degeneration the cytoplasm of these cells becomes intensely oxyphilic.

That the globules are not single, or fused masses of, red blood-corpuscles is here even more definitely indicated than in the case of the osteoclasts. Blood-vessels filled with erythroplastids abut upon the enamel organ, so that it is readily possible to bring such a vessel and a portion of the pulp with globules into the same field, when careful comparisons can be made between the two. No blood-vessels can be seen in my preparations entering the enamel organ.[1] The erythroplastids differ in shape, size,

[1] I am unable to confirm the conclusion of Hopewell-Smith that the enamel pulp (stratum intermedium) of the kitten's tooth is supplied with a capillary plexus ("Normal and pathological histology of the mouth," vol. 1, pp. 263–264). His figures 227 and 229, supplied to illustrate his description of a vascularized pulp, do not obviously bear such interpretation. As near as one can determine from these photomicrocraphs, the capillaries end abruptly on the outer surface of the enamel organ, as in my preparations. Hopewell-Smith introduces this

and staining reaction from the 'osseous globules' in the same manner as above described for the alveolar marrow. It must be added, however, that many of the pulp globules have a sort of brownish-gray, or less deeply bluish-red, color than those of the osteoclasts. This difference may signify a later stage in resorption in the case of the pulp globules. That the globules also in the enamel pulp do not signify hyaline degeneration seems definitely indicated by the generally healthy character of the nuclei of the pulp syncytium.

The crucial point concerns the origin and significance of these pulpar osseous globules. Two possibilities are presented: 1) that they represent decalcified resorbed enamel; 2) that they represent a superfluous enamel secretion on the part of the cells of the enamel pulp, which is then resorbed through the agency of newly formed polynucleated giant-cells. The latter possibility has a color of reasonableness, since the cells of the enamel pulp are originally very similar to those of the adjacent ameloblasts and would accordingly be expected to have similar functional capacities. It is difficult definitely to dispose of this suggestion. There is, however, one datum of at least apparent countervailing significance: large elongated globules occur between the inner border (stratum intermedium) of the enamel organ and the definitive enamel. Moreover, the border of the enamel in such regions appears uneven, and occasionally even jagged, as if enamel were being dissolved peripherally. It would seem probable, in view of this observation, that the newly

subject of the blood-supply of the enamel organ with the statement that "The vascularity or otherwise of the enamel organ is not yet determined, many competent authorities holding opposite opinions on this subject. Thus Lionel Beale, Leon Williams, Howes, and Paulton assert that a vascular network is to be found in the stratum intermedium, while Tomes, Paul, Andrews, Wedl, Sudduth, and Magitot affirm its non-vascularity.

"The author, in a joint paper with H. W. Marett Tims, has recently described the presence of blood-vessels, containing erythrocytes in the enamel organ of the Australian wallaby. ('Tooth germs in the wallaby, Macropus billiardieri'; Proc. Zoolog. Soc., London, 1911)" (pp. 261–262). He then proceeds to describe "The blood supply of the developing dental tissue" in Mammalia and illustrates his description as regards the vascularization of the enamel pulp with a "coronal section through the mandible of a kitten" (pp. 259 and 264), which he interprets as showing pulp capillaries.

formed enamel becomes to some extent resorbed and reshaped peripherally before the deciduous tooth is finally erupted. But in the event that the latter should prove the correct interpretation of the presence of these globules within the enamel pulp, I am quite unable to decide, on the basis of available data, as to whether the active agent of resorption is here the enamel pulp as a whole or specifically the giant-cell (adamantoclast, ameloclast). That the blood-vessels have nothing directly to do with the process of decalcification and resorption of the enamel seems clear from the fact that they are excluded from the enamel organ. But it is to be recognized that blood-vessel intervention in the process of resorption might be viewed as simply the obverse of the logically assumed nutritive rôle of the blood-vessels with respect to enamel elaboration. All things considered, I incline to the view that these globules of the enamel pulp are superfluous secretion products of slightly active potential ameloblasts of the stratum intermedium of the pulp, and that the formation and presence of multinucleated giant-cells signifies the fulfillment of a demand for the removal of the globules by phagocytosis. But the alternative view that the globules are derivatives of the definitive enamel, resorbed either through the agency of the pulp as a whole or specifically through the agency of the newly formed giant-cells is not disproved. Under either view, however, the pulpar giant-cells are ameloclasts in the sense that they ingest and destroy enamel.

The resemblance, or apparent chemical identity, between the globules ingested by the osteoclasts and those of the ameloclasts results from the essential chemical identity between bone and enamel. While the evidence is fairly conclusive that the osteoclasts are the active specific agents in the resorption of bone in these specimens, it warrants for the present only the assertion that the giant-cells of the enamel pulp ingest and destroy enamel globules derived from an unknown source by action of an unknown agency. This source may obviously be either the newly formed enamel or the cells of the pulp syncytium. The obvious active agent in resorption may be either the enamel pulp or the newly formed giant-cells. Less obvious sources and factors cannot, however, at present be definitely excluded.

As regards the osteoclasts, two facts stand in sharp contradiction to Arey's (*1*) conclusion that they should be interpreted as degenerating fused osteoblasts: 1) Osteoclasts arise in large part directly from the connective tissue stroma of the marrow, independently of osteoblasts; 2) the younger osteoclasts exhibit no signs of degenerative changes, either cytoplasmic or nuclear.

SUMMARY

In the mandible of the newborn cat occur many multinucleated giant-cells which contain a variable number of spheroidal globules of a material apparently identical with bone. These giant-cells occur both in the marrow of the developing jaw bone and in the enamel pulp of the developing tooth. Both groups of cells arise in a similar manner; in one case by fusions among the cells of the marrow reticulum, in the other case by fusion of cells of the dental pulp. In respect of the mode of origin, and of their function, these cells accordingly resemble foreign-body giant-cells. The former are osteoclasts, the latter ameloclasts; one group ingests and destroys globules of bone, the other globules of enamel. The fundamental factors operating in the decalcification and dissolution of the bone and of the enamel remain unknown. In the case of the osteoclasts, however, the evidence suggests that the giant-cells are the active specific agents in the dissolution of the bone, as well as in the elimination through phagocytosis of the products of dissolution. The fact that in many other locations where resorption of growing bone occurs, similar giant-cells are very numerous, though without osseous inclusions, is in accord with this view. The presence or absence of the products of resorption in the shape of globules, in the cytoplasm of specific osteolytic giant-cells, may be correlated with the relative rapidity, or perhaps the peculiar mode, of resorption of the bone. The products of osseous resorption of specific osteolytic giant-cells, generally of ultramicroscopic size, may under certain conditions be of microscopic size, like the globules here described for these osteoclasts and ameloclasts.

LITERATURE CITED

1 Arey, L. B. 1920 The origin, growth, and fate of osteoclasts and their relation to bone resorption. Am. Jour. Anat., vol. 26, pp. 315–347.

2 Billroth 1869 Allg. chirurg. Pathol. und Therapie. 4. Aufl., S. 195 (cited from Koelliker (6)).

3 Hopewell-Smith, A. 1918 Normal and pathological histology of the mouth. Vols. 1 and 2. P. Blakiston's Son & Co., Philadelphia.

4 Jordan, H. E. 1918 A contribution to the problems concerning the origin, structure, genetic relationship, and function of the giant-cells of hemopoietic and osteolytic foci. Am. Jour. Anat., vol. 24, pp. 225–269.

5 Koelliker, A. 1872 Weitere Beobachtungen über das Vorkommen und die Verbreitung typischen Resorptionsflächen an den Knochen. Verh. d. phys.-med. Gesellsch. in Würzburg. N. F. Bd. 3, S. 215–228; and Archives de Zoologie expérimentale. T. 2, pp. 1–28 (1873) (cited from Koelliker (6)).

6 1873 Die normale Resorption des Knochengewebes und ihre Bedeutung für die Entstehung der typischen Knochenformen. Vogel, Leipzig; S. 86.

7 Tomes and de Morgan 1852 Observations on the structure and development of bone. Phil. Trans. (cited from Koelliker (6)).

PLATES

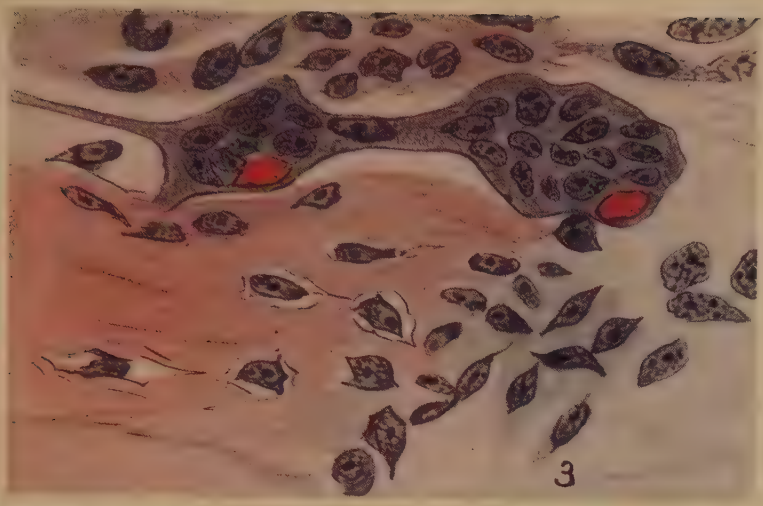
PLATE 1

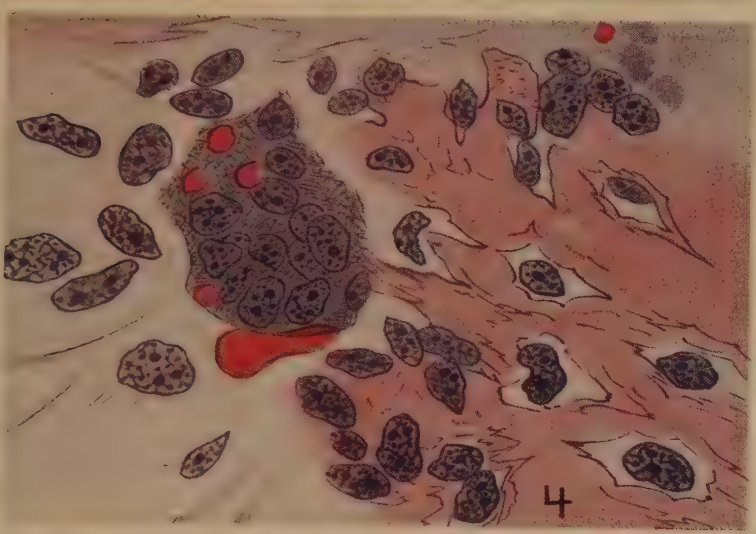
EXPLANATION OF FIGURES

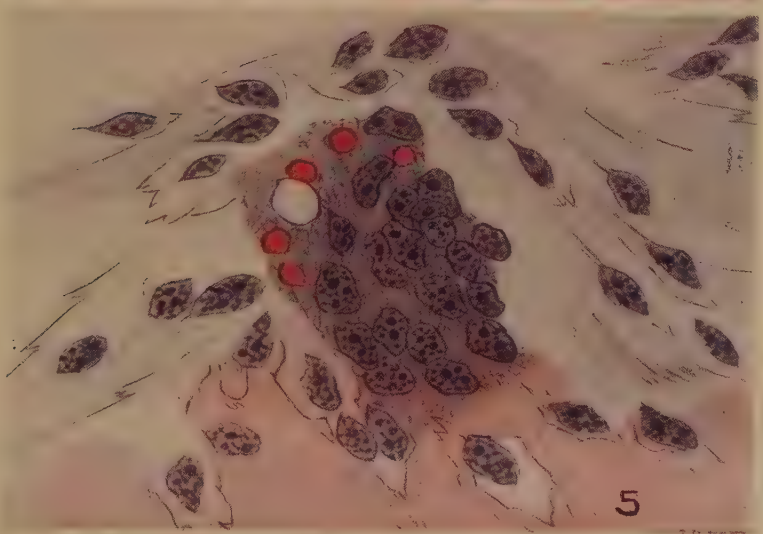
3 Large bilobed osteoclast, containing two large globules of osseous material. The osteoclast rests upon a spicule of bone. × 825. Drawings by Miss Emma M. Whitfield, Richmond, Virginia. (The three water-color illustrations, and the corresponding microscopic preparations, were shown as demonstrations at the meetings of The American Association of Anatomists, in Washington, April 1 to 3, 1920.)

4 Osteoclast, at the left of the spicule of bone, containing four smaller osseous globules. Along the lower border of the giant-cell lies a large pestle-shaped mass of osseous material in process of ingestion. × 825.

5 Osteoclast, continuous below with several osteoblasts, covering the spicule of bone. It contains five small deeply staining globules, and one larger lighter-staining globule. The latter probably represents a large osseous globule at a later stage of resorption. × 825. (The osteoclasts of figs. 3, 4, and 5 are from the marrow areolae of the subdental bone, corresponding to that along the lower margin of fig. 1.)







PRESS WORK BY FRED'K GOEB

Resumen por los autores, Paul K. Webb y J. Barrett Brown, Universidad Washington, Saint Louis.

Un caso de barras costales independientes en e lepistrofeo del hombre.

En el presente trabajo se dá a conocer la existencia de un par de procesos costales independientes en el epistrofeo de un varón negro. Las barras costales consisten en dos osículos independientes de un centímetro y medio de longitud, y del mismo tamaño, que se articulan por medio de una anfiartrosis con protuberancias cortas que se extienden desde el cuerpo del axis. Estas protuberancias ocupan la posición del extremo proximal de la barra costal normal. Las barras costales independientes se extienden inferiormente y lateralmente, sin fundirse con los pedículos posteriores o con los verdaderos procesos transversos. Los orificios costo-transversos son por consiguiente incompletos, abriéndose hacia los lados. En el atlas no existen barras costales; los cuerpos y arcos de la segunda y tercera vértebra son continuos en cierto grado, y esto es probablemente una condición secundaria.

Translation by José F. Nonidez
Cornell Medical College, New York

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, JANUARY 17

A CASE OF INDEPENDENT COSTAL BARS OF THE EPISTROPHEUS IN MAN

PAUL K. WEBB AND JAMES BARRETT BROWN
Department of Anatomy, Washington University

TWO FIGURES

This rare variation was discovered in the class laboratory during the preparation of the vertebral arteries. The subject was a male negro of advanced age, as indicated by extreme emaciation and wrinkled skin, sparseness of hair, toothless jaws, and fragile bones. The record taken from the mortuary report gave his age at ninety-five years.

On tracing the vertebral arteries through the transverse foramina toward the cranium, our attention was arrested on reaching the epistropheus by the mobility of the two costal bars of that vertebra. Examination disclosed two ossicles, similar in form and of nearly equal size, articulating, one on either side, with the body of the epistropheus (figs. 1 and 2). Each bone presented the shape of a little horn, directed downward, backward, and a little laterally, tapering below to a free blunt extremity. The ossicle is bent into a well-marked curve, convexity forward. That of the right side measured 9 mm. in length and 4 mm. in the greatest diameter; the left bone, 10 mm. and 4 mm. They occupied the usual position of the costal component of the cervical transverse process and were therefore in series with the costal bars of the succeeding vertebrae. The ossicles do not have a true capitular termination against the body of the epistropheus, but articulate with a truncated process springing from the base of the root of the arch just beneath the superior articular surface. A careful preparation of the joint revealed an amphiarthrosis, the articulating surfaces being covered with cartilage, without, however, a joint cavity or synovial layer. The union was maintained by fibrous tissue apparently in con-

nection with the periosteum and tendons of muscles. As already intimated, the joint permitted a small degree of motion of the ossicle. The opposite extremity of the latter ended free, did not articulate, or even come into contact with the truncated process representing the posterior bar of the transverse process. The transverse foramen was therefore open laterally.

Other abnormalities were subsequently discovered more or less closely related to that just described. The lamina and inferior articular process of the left side of the epistropheus are continuous with the lamina and the superior articular process of the third vertebra; bony union also obtains between the third and fourth vertebrae in two regions, viz., between the articular processes of the right side and between the bodies on the left. Some degree of thickening of the articular processes and laminae is noticeable where the union exists. The union between the bodies of the third and fourth is marked by a bony callosity. A certain amount of deformation of the bodies of the fourth and fifth vertebrae, affecting the anterior opposed margins, is evident.

The atlas also presents a variation. This rudimentary bone is still further reduced by the absence of its anterior costal bars. In place of them, there was found on each side a slender ligament completing the transverse foramen anteriorly. This band was stretched between the lateral free extremity (tubercle) of the transverse process of the atlas and a prominent, short, thick process of the lateral mass opposite the front of the superior articular surface. This may be a vestige of the costal bar or a capitular process.

As to the occurrence of like variations, it is of course well known that absence of the costal bar of the atlas has been frequently observed; Macalister ('93), Bolk ('99), and Le Double ('12) in particular have described and discussed the significance of this variation. All degrees of defect of this bar, ranging from nearly its normal size to complete absence, have been noted.

Elliot Smith ('07) has described a case of fusion of the atlas and axis in which there was no evidence of disease. In the present case the cause of fusion between the laminae and articular processes of the epistropheus and third vertebra cannot

with certainty be referred to an inflammatory process; this is probably the cause. The deformation and ankylosis of the bodies are evidently the result of disease; the irregular contour is in contrast with the smoother surface where the axis and third vertebra are continuous by their articular processes. If the latter union were primary, it is not improbable that the fixation of the joints between these bones might have been a predisposing factor in the location of the disease process in the joints of the succeeding vertebrae. The phenomenon of coalescence of vertebrae both as a normal ontogenetic process and as a not uncommon variation is well established by observation: fusion of the cervical vertebrae to constitute a single piece, comparable to the status of the sacrum in man, is normal among cetaceans; there is marked tendency toward fusion of the atlas with the occipital bone in man, as shown by the descriptions of many cases (Dwight, '09; Kollmann, '04; Swjetschnikow, '06, and others). In a large percentage of such cases there is no evidence of disease as an influence in bringing about the fusion.

Regarding the occurrence of independent costal bars in the cervical region, a very large literature has grown up chiefly from interest in cervical ribs. The known cases of cervical ribs have been collected and reviewed by Le Double and additional facts have been brought to light by the more recent work of Todd ('12). The usual type of cervical rib is connected with the seventh vertebra; a rare association is that with the sixth. Szawlowski ('01) has reported a case of costal rudiments in connection with the fourth bone. Macalister ('94) has found an independent bony granule in the pre-arterial crus of the transverse process of the axis. There is evidence, according to Fawcett ('10), of independent chondrification of the costal bars. So far as we know, no cases of vestigial ribs have been found in connection with the atlas, epistropheus, and fifth vertebra. Consideration of all the conditions in the present case lead us to the conclusion that the independent ossicles have developed from centers separate from the ossific processes of the epistropheus and by their relations must be regarded as vestigial ribs. The ossicle represents probably only the neck of a rib, since it

does not reach the true transverse process and at its vertebral end articulates with a prominent process (capitular?) without, however, expanding to form a head. It is comparable to the rib rudiments in the cephalic part of the cervical region of reptiles.

The chief interest of this case, it seems to us, lies in its possible connection with the tendency to regression manifested in so many directions by the components of the head-neck region. Bolk (loc. cit.), who has studied this phenomenon, points out the evidences of this reaction in the reduced state of the atlas and its proneness to further degeneration and assimilation with the occipital; the imperfection of the first spinal nerve correlated with the absence of muscles in the intertransverse and interspinous series, etc. Further consideration of this question has been given by Terry ('17) in connection with the development of the occipital region of the chondrocranium. The present case exhibits a group of changes, differing in kind (defect of atlas, coalescence of vertebrae, abortive development of epistropheus), which may, nevertheless, be the result of a single factor operating upon the formation of this transitional zone between the head and neck.

The authors wish to express their appreciation of the assistance given them by Dr. R. J. Terry in the preparation of this report.

LITERATURE CITED

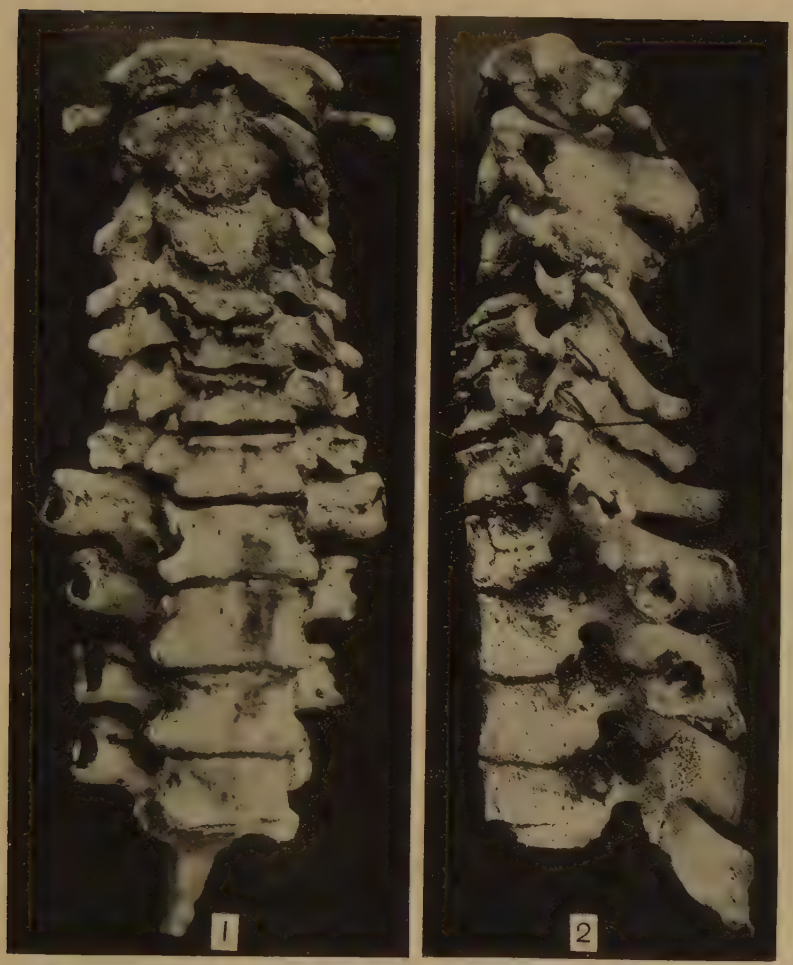
Bolk, L. 1899 Neder. Tijdschr. voor Geneeskunds, vol. 1.
Dwight, T. 1909 Anat. Rec., vol. 3.
Fawcett, J. M. 1910 Journ. Anat. and Physiol., vol. 44.
Kollmann, J. 1904 Anat. Anz., Bd. 25.
Le Double, A. 1912 Traité des variations de la colonne vertébral. Paris. Vigot.
Macalister, A. 1893 Jour. Anat. and Physiol., vol. 32.
1894 Jour. Anat. and Physiol., vol. 28.
Smith, G. Elliot. 1907 Anat. Anz., Bd. 31.
Swjetschnikow, W. A. 1906 Arch. f. Anat. u. Physiol.
Szawlowski, J. 1901 Anat. Anz., Bd. 20.
Terry, R. J. 1917 Jour. Morph., vol. 22.
Todd, T. W. 1912 Jour. Anat. and Physiol., vol. 46.

PLATE 1

EXPLANATION OF FIGURES

1 Independent costal bars of the epistropheus in man. Cervical and upper thoracic spine from in front. $\times \frac{3}{5}$.

2 Independent costal bars of the epistropheus in man. Cervical and upper thoracic spine from the left side. $\times \frac{3}{5}$.


1
2

Resumen por F. S. Hammett, por el autor Vincent Vermooten, Universidad del Cabo, Africa del Sur.

Estudio de la fractura del epistrofeo en el ahorcado, con una nota sobre las causas posibles de muerte.

En el presente trabajo el autor presenta una breve descripción anatómica de la naturaleza de las fracturas cervicales observadas en cuatro casos de ejecución judicial en la horca. En todos los casos ha encontrado una dislocación de la segunda y tercera vértebra, en dirección inferior. La primera y segunda vértebra estaban todavía articuladas correctamente, así como el atlas con el occipucio. Puesto que el ligamento transverso conservaba todavía su posición normal, mientras que el arco neural estaba desprendido de la vértebra, el autor cree que este es el resultado ordinario después de morir ahorcado y, por consiguiente, no está conforme con la opinión de Gray, que admite la ruptura del ligamento transverso en tales casos. Vermooten cree que la muerte se debe probablemente a la rotura de la médula.

Translation by José F. Nonidez
Cornell Medical College, New York

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, DECEMBER 27

A STUDY OF THE FRACTURE OF THE EPISTROPHEUS DUE TO HANGING WITH A NOTE ON THE POSSIBLE CAUSES OF DEATH

VINCENT VERMOOTEN

Anatomical Laboratory of the University of Cape Town, South Africa

TWELVE FIGURES

The cause of death due to hanging (judicial hanging), although a very interesting phenomenon, has apparently not been the result of much investigation. It has usually been taken for granted that death is due to rupture of the transverse ligament of the atlas with the resulting crushing of the cord and medulla by the odontoid process of the epistropheus. That this is not always the case is shown by the examination of four cases of hanging which the author investigated in this laboratory during the latter part of the year 1919.

On examination it was found that in all the four cases there was a downward dislocation of the second and third cervical vertebrae. The first and second vertebrae were still properly articulated as well as the atlas with the occiput, the ligamentum apicis dentis being still firmly attached to the occiput and to the tip of the odontoid process of the epistropheus.

Another remarkable fact was revealed: the transverse ligament of the atlas was still in its normal position, holding the odontoid process as in life. There was not even the faintest trace of a rupture of the ligament in any of the four cases.

In the first cases (figs. 1, 2, 3,) the atlas and the epistropheus were well articulated, as were the atlas and occiput. The epistropheus and third cervical vertebra were dislocated downward, while the transverse ligament of the atlas was intact.

The epistropheus, strangely enough, was fractured, the neural arch being altogether broken off from the body. The fracture

on the right side was posterior to the foramen transversarium, but anterior to the inferior articular facet. On the left-hand side the fracture was through the pedicle and through the trans-

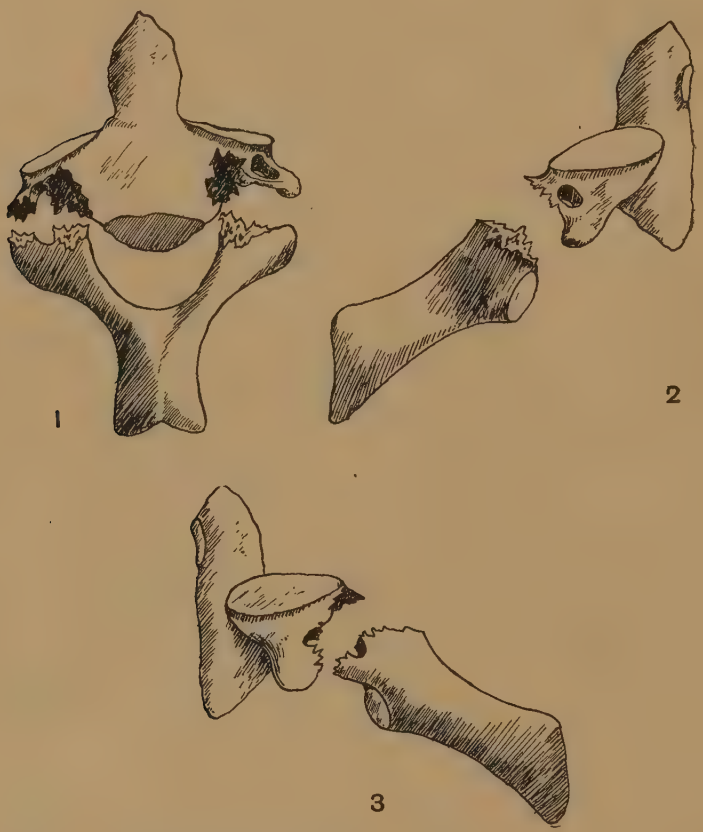


Fig. 1 Body and neural arch of vertebra.
Fig. 2 Portions of vertebra viewed from the right side.
Fig. 3 Portions of vertebra viewed from the left side.

verse element of the transverse process, leaving the greater part of the foramen transversarium anterior to the break.

In the second case (figs. 4, 5, 6) the vertebrae were again articulated as in the first case, with the transverse ligament of

the atlas altogether intact. The neural arch was also fractured on both sides. On the right-hand side the break was anterior to the inferior articular facet and posterior to the foramen transversarium, leaving the foramen quite whole. On the left-hand side there was a break in the same position as on the right, but the sides of the foramen transversarium were also broken so that the transverse process was altogether broken off from the vertebra.

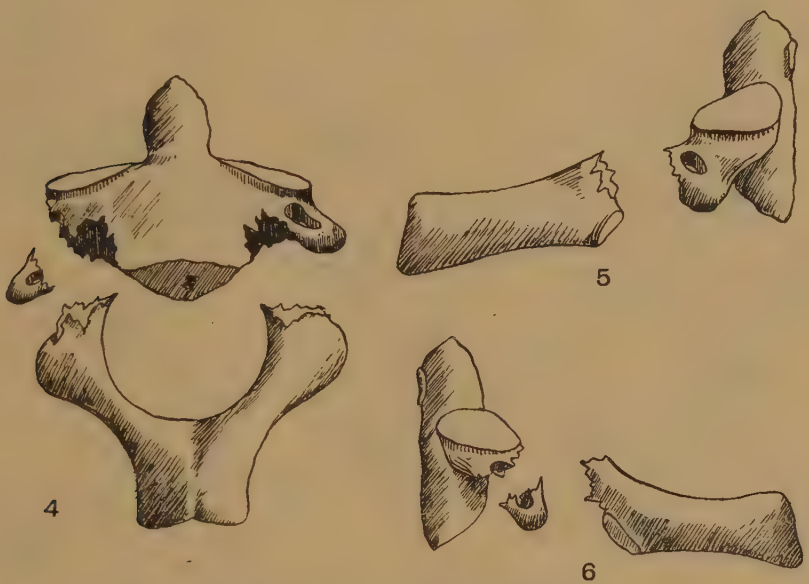


Fig. 4 Body and neural arch of the vertebra.
Fig. 5 Portions of vertebra viewed from the right side.
Fig. 6 Portions of vertebra viewed from the left side.

In the third case (figs. 7, 8, 9) once more the conditions of articulation were as in the previous cases, with absolutely no sign of rupture of the transverse ligament of the atlas and the neural arch was once more totally broken off from the body of the vertebra.

The fracture on the right-hand side was in exactly the same position as the fracture on the right-hand side of the second case. On the left-hand side the fracture was much more serious, for,

in addition to a fracture similar to the one on the right-hand side, there was also a fracture through the pedicle and through the junction of the posterior third and anterior two-thirds of the superior articular facet, anterior to the foramen transversarium. A large part of the arch containing the foramen transversarium was thus broken from the lamina on the one hand and from the body of the vertebra on the other.

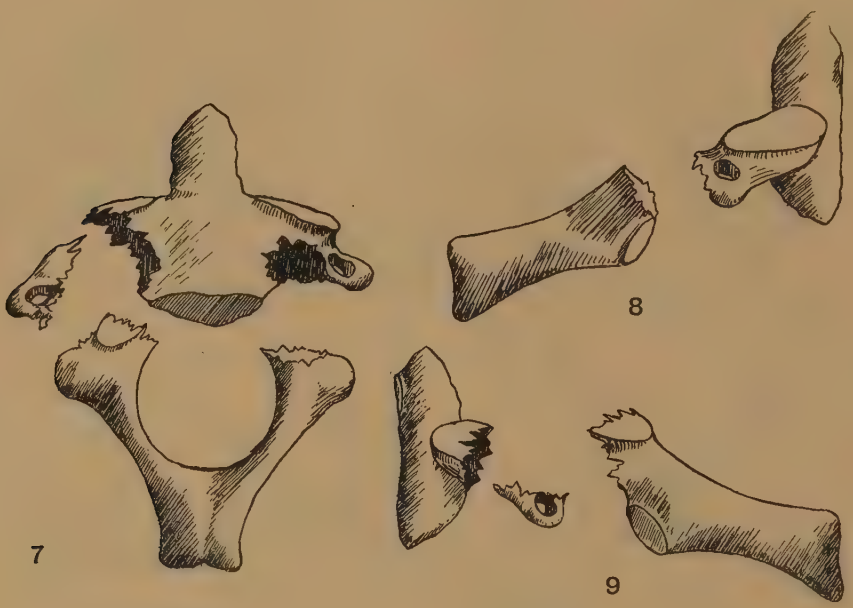


Fig. 7 Body and neural arch of the vertebra.
Fig. 8 Portions of vertebra viewed from the right side.
Fig. 9 Portions of vertebra viewed from the left side.

In the fourth case (figs. 10, 11, 12) the articulations and the condition of the transverse ligament of the atlas were again exactly similar to the previous three cases, the break on the right-hand side was also similar. On the left-hand side the break varied again; this time the break was through the costal element of the transverse process, through the foramen transversum and through the posterior part of the superior articular

surface. In addition to this, it was found that the atlas was also mutilated, the transverse process being broken off from the lateral mass, the fracture being through the roots of the transverse and costal elements. The transverse process itself was also broken into three pieces.

In Gray's Anatomy, page 358, we find the following statement:

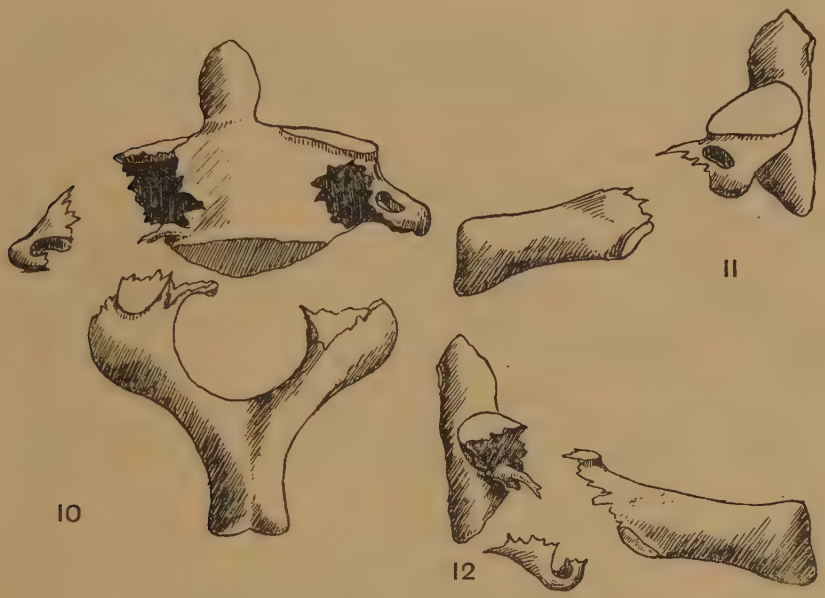


Fig. 10 Body and neural arch of vertebra.
Fig. 11 Portions of vertebra viewed from the right side.
Fig. 12 Portions of vertebra viewed from the left side.

Dislocation of the occipital bone from the atlas has been recorded only in one or two cases; but dislocation of the atlas from the epistropheus, with rupture of the transverse ligament of the atlas, is much more common: it is the mode in which death is produced in many cases of execution by hanging.

From this it appears that rupture of the transverse ligament is a fairly ordinary procedure, especially in cases of death due to judicial hanging. On examination, however, of four cases of

death by hanging it was found that not only was the transverse ligaments of the atlas not ruptured, but there was not the faintest sign of a rupture, the whole ligament was intact, including even its attachment to the atlas; the dens of the epistropheus being held as though it were the true body of the atlas.

Treves and Keith, in their "Surgical Applied Anatomy," page 645, say:

> In the atlanto-axial region the amount of displacement that follows upon luxation of the two bones from one another is such that the cord is, as a rule, severely crushed, and death ensues instantaneously, as is seen in cases of death by hanging.

The crushing of the spinal medulla is here also evidently attributed to the odontoid process.

It may be that occasionally the transverse ligament ruptures on hanging and that under these circumstances the cord is crushed and causes instantaneous death, but in these cases the ruture is most likely due to congenital weakness of the transverse ligament or perhaps to a pathological condition of the transverse ligament or atlas. In the cases of the four well-built young colored men, it is not possible to ascribe death to rupture of the transverse ligament.

Death would be largely due to rupture of the cord, which is allowed for by the downward dislocation of the third cervical vertebra from the second. It is difficult to say where the rupture actually takes place, for the specimens were examined long after the postmortems had been held. To what other cause instantaneous death can be due is difficult to say, as crushing of the cord, as mentioned by Treves and Keith, does not seem very probable, for nothing projects into the canal. The elasticity of the transverse ligament may allow for an instantaneous, slight protrusion of the odontoid process into the neural canal, but this small amount can be compensated for by the fact that the cord is suspended in the neural canal. It is difficult to say what part the fracture of the epistropheus plays. Perhaps this also aids in the crushing of the cord.

In addition to the probable rupture of the cord, one must also take into account the rupture of the big blood-vessels of the brain and vertebral arteries in addition to shock and the rupture of several of the muscles of the neck, such as the M. sternohoideus, M. omohoideus, and others, as well as the possible rupture of the carotid arteries.

LITERATURE CITED

GRAY Anatomy, Descriptive and Applied.
TREVES AND KEITH Surgical Applied Anatomy.

Resumen por el autor, Paul E. Lineback,
Universidad Emory, Atalanta, Georgia.

Un caso de polidactilia en un embrión de 22 mm.

El caso presentado en este trabajo, si el autor está bien informado, es el más jóven mencionado hasta el presente, debiendo su significación principalmente a este hecho. Además susministra datos adicionales intesantes sobre el problema del primer hueso metacarpiano y sobre el problema más general del polidactilismo. En este caso el autor encuentra, sin embargo, pocas pruebas concluyentes en favor de las teorías relacionadas con ambos problemas. El único punto valioso que el autor puede ofrecer es que el presente caso lleva el problema del polidactilismo desde el catálogo de los factores causativos extrínsecos al dominio de los factores formativos tempranos.

Translation by José F. Nonidez
Cornell Medical College, New York

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, FEBRUARY 7

A CASE OF UNILATERAL POLYDACTYLY IN A 22-MM. EMBRYO

P. E. LINEBACK

Emory University School of Medicine

ONE FIGURE

In the laboratory of the author there is an embryo 22 mm. in length, with an extra digit on the right hand at the base of the metacarpus of the thumb. It was noted by Doctor Streeter while making measurements of the embryo, and he marked it as being, from all available records, the youngest case thus far discovered. This fact seems to warrant the author in making a study and report of it, with a brief survey of the literature on the subject.

The embryo came into the laboratory without data as to its parents or details of its appearance. It has a crown-rump length of 22 mm. and is well formed in every detail, with membranes in normal state. The two hands are similar in every respect, except that the thumb on the right hand is a trifle smaller than the left thumb. The breadth of each hand, midway across the palm, is 2 mm., and the length, from a line across the wrist just back of the thenar eminence to the tip of the middle finger, is 2.5 mm. The right thumb is 1.1 mm. long and 1 mm. thick. Attached to the base of its metacarpus is the extra digit. It stands out from the surface at right angles and is uniform in size and shape. Near the tip it bends slightly forward and terminates in a blunt rounded point. It is 1 mm. long and 0.6 mm. thick. Serial cross-sections were made of the whole forearm and hand beginning at the tip of the fingers and going to the elbow. The sections were cut 10μ thick after staining the specimen in toto in cochineal.

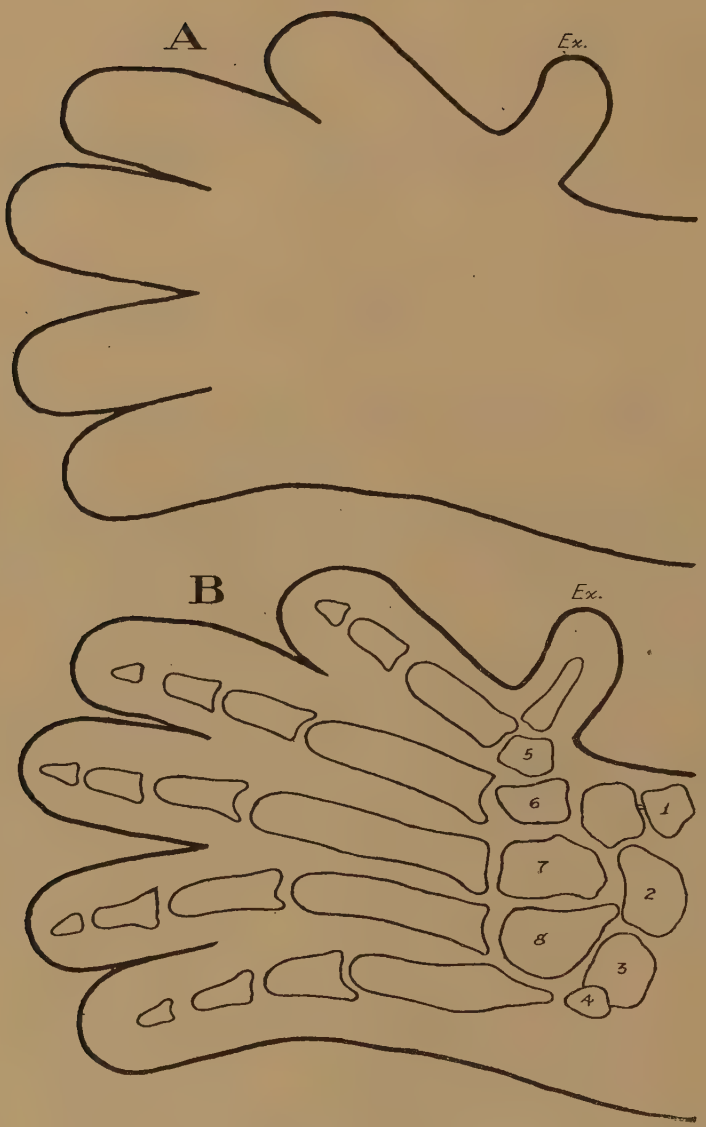


Fig. 1 Outline drawing from reconstructed model. × 22. A. Surface view showing position of extra digit. B. Cartilaginous skeleton showing relationship of extra digit to other skeletal units

Microscopic study shows the cartilages of the wrist, hand, and fingers present. All the separate units of the parts are in place and are still in process of cartilage formation. Each piece shows a peripheral zone of condensed cells beginning to show the features of a perichondrium, and a central core characterized by a network of hyaline strands, in the meshes of which may be seen large cartilage cells. The skeleton of the extra digit is a single piece of cartilage having the above characteristics. It is a separate unit with close relationship to the base of the metacarpus, and not an offshoot or spur from it.

Two muscles are contained in the digit, a volar and a dorsal muscle. The one on the dorsal side extends through the mesenchyma out toward the tip, but ending short of it. Its origin and identity are uncertain; probably it comes from the outer head of the first dorsal interosseous muscle. The volar muscle can be more certainly identified. It arises from the abductor policis brevis and extends to near the tip of the digit on the palmar side. Nerves cannot be identified and vessels are seen only as undifferentiated capillaries.

In going through the literature, no cases are cited of polydactyly in the young embryo; all are late foetuses or postnatal cases and the majority are in adult life. Salzer ('98), reporting two cases of triple phalangeal thumbs, opened the question as to which phalanx is missing in the ordinary thumb, the end phalanx or the middle phalanx. He quoted Windle, who made a general summary of such cases by expressing the opinion, with some reservations, that the extra phalanx must have occurred from the fact that the distal bone center of the first metacarpus developed independently. He referred to Uffelman's work, in which it is shown that at the capitellum of the first metacarpus a special bone center is present without ever existing independently. He thinks that this extra center may give rise to a supernumerary phalanx, which would be the main phalanx. Salzer quoted Pfitzner also, who seemed to hold that a triple phalangeal formation is, for a brief period, a normal state and that the double phalangeal thumbs and toes have come about in this manner, from the merging of the middle and end phalanges

gradually a typical and enlarged phalanx has resulted. In support of this he cites a case of a girl with a strikingly large end phalanx consisting of two pieces which have not been completely fused. Jurcie ('06) reported a case of hyperphalangea in both thumbs. In this report he took up the problem of whether the thumb should be considered as possessing normally three phalanges and no metacarpus, or a true metacarpus and only two phalanges. He cites the fact that the first bone of the thumb develops in the same manner as the phalanges, the head and shaft from a common primary center, and the base from an epiphysis. The development of the metacarpals is just the reverse, the base and shaft arise from a common primary center and the head from an epiphysis. He concludes, however, that from the manner of muscle attachment to the first metacarpus and noting the presence of the extra ossification center in some mammals, and occasionally in man, this is a true metacarpus and not a basal phalanx.

Stieve described a case of bilateral hyperphalangea ('15–16) and quoted Krause ('09), who objected to Pfitzner's work on the ground that it dealt with malformations, which was poor material for drawing such conclusions. In Stieve's case the end phalanx, which should be shorter than in normal diphalangeal thumbs, is especially long, and in Salzer's cases the two end phalanges show no proportionate shortening. He finds also that whereas there was restricted movement between the end and middle phalanges of Hilgenreiner's case, the movement in his case was especially free. Backed by these points, he comes to the conclusion, differing with his predecessors, that the end phalanx in the diphalangeal thumb is not a fusion of the middle and end phalanges. He seems to hold to this opinion more firmly when he notes that we are not dealing with perfect triphalangeal thumbs in reported cases, but the extra piece is only the radial side of the middle phalanx.

Hilgenreiner apparently has the most complete list of cases of hyperphalangeal thumbs and the most valuable data concerning the subject. According to him, 107 three-jointed thumbs, fifty-eight cases, had been noted to date ('10). He goes to

some length in discussing the condition and makes a definite classification of the cases. In the first class he would include cases where there is only partial separation of the end and middle phalanges—incomplete hyperphalangea. In class 2 he would include only such cases as show complete formation of the middle phalanx having a diaphysis and an epiphysis—true hyperphalangea. In these cases the thumb characteristics have been lost and the structure is more like an extra index-finger. This change of characteristics is proportionate to the degree of separation of the phalanges. He is quite firm in his opinion that the condition of diphalangeal thumb has arisen from a fusion of the end and middle phalanges in early stages of formation. He sees two possible explanations in these cases of anomalous thumbs, but does not pursue either at length. He speaks of a palingenesis of the middle phalanx traceable to some endogenous cause. This factor is mentioned by others also, Minoura and Prentiss. He also states that the supernumerary phalanx is not always to be explained as a palingenesis of the middle phalanx, since it may arise from the epiphysis of the second phalanx. In such cases triphalangeal thumbs appear only postpartem in the first, second, or third year of child life.

Prentiss ('03) has contributed to the subject a valuable piece of work, dealing with it in a somewhat broader fashion than Hilgenreiner, although his study of cases in man, especially of thumb variations, is rather too limited for valuable application here. He states that "variations are found chiefly in digits which are modified, rudimentary or vestigial." As to the causes of polydactyly he has this to offer: "in man it is not a definite number of extra digits but a tendency to duplication which is inherited" and "duplication of functional digits is probably caused by germinal variations alone."

After reviewing the above literature, which is, although brief, a general and inclusive statement of opinions on the subject, several problems present themselves or at least several aspects of the same problem. There appears to be a difference between hyperphalangea and polydactyly. The former is characterized by the presence of an extra phalanx, with the main disfiguration

of the thumb an increase in its length or a deflection to the ulnar side. The latter is marked by the presence of an extra digit, either large or small, fully formed or rudimentary, which stands apart from the other digits. The problem of di- and triphalangeal thumbs, as to how the normal thumb should be regarded and the origin of the extra phalanx in triphalangea, comes under the head of hyperphalangea. Here would be placed the cases of Jurcié, Salzer, Stieve, and some of Hilgenreiners. Under polydactyly would be included such cases as Prentiss', others of Hilgenreiner's, and the author's.

Another point is that variations in the thumb should be considered in a special class apart from variations in the other fingers, since here is found, in the normal state, a condition unlike that in the others.

Prentis's statement that, "variations occur chiefly in digits that are modified," would lend emphasis to this, and Hilgenreiner seems to have considered it worth while to tabulate over one hundred cases of thumb variations.

Standing out prominently among these points is the problem of the causative factor in polydactyly and hyperphalangea, and how this factor operates. Upon this point the author's case presents some features, not noted in others, which might be considered a little more at length. Here is a clear case of polydactyly having an extra digit with a separate piece of cartilage. All the other units of the wrist and hand are present but still in the cartilaginous state. It is conclusive that the causative factor of splitting of a digit by an amnionic band is not operative here. Nor is the factor of an extra phalanx arising from a diverted center of ossification to be applied, since the digit is present before ossification appears anywhere in the hand. The specimen permits of an investigation further back than the beginning of ossification. Here might be applied the theory of palingenesis as suggested by Minoura, Hilgenreiner, and Prentiss, although the last author might not include it here. Or again, Prentiss' reversion to a not remote ancestral form, an inheritance, working through Mendel's law, might be applied. He states that "all cases of polydactyly, not due to external

causes, may be the result of inheritance." These two theories presuppose a parental state from which they may work, and the natural question arises as to what caused the first deviation in the original parent. 'Germinal variation' is an expression commonly used by investigators and especially by Prentiss when he says, "but the duplication of functional digits are probably caused by germinal variation alone." He further states that, "as to the cause of these germinal variations . . . . we know little or nothing." In his comprehensive work, "Science and philosophy of the organism," Driesch put the essence of the matter in a more concrete form when he gave expression to 'feeling for form.' Just how far these expressions are usable is a question; they at least emphasize the fact that search must be made in the field of cytology and cytochemistry in dealing with the state of abnormal thumbs.

CONCLUSIONS

The author feels justified in drawing the following conclusions:

1. Hyperphalangea and polydactyly are sufficiently different to be put into separate classes.

2. The thumb offers sufficient significance to warrant separate study in its variations.

3. The author's case offers proof that some cases of polydactyly owe their origin to earlier causes than external factors or deviation of ossification centers.

LITERATURE

DRIESCH Science and philosophy of the organism, vol. 1.

HILGENREINER, HEINRICH 1915–16 Über Hyperphalangea des Daumen. Ant. Anz., Bd. 48, S. 565.

JURCIÉ, F. 1906 Ein Fall von Hyperphalangie beider Daumen. Lagenbeck, Arch. f. Klin. Chir. Bd. 80, S. 562.

MINOURA, MITSUO 1910 Ein Beitrag zur Kenntnis der Missbildungen an dem Extremitäten. Inaug. Diss. München.

PRENTISS, C. W. 1903 Polydactylism in man and the domestic animals with special reference to digital variations in swine. Bull. Mus. Comp. Zöol., Harvard, vol. 40, no. 6.

SALZER, HANS 1897–98 Zwei Fällen von dreigliedrigen Daumen. Ant. Anz., Bd. 14, S. 124.

STIEVE, H. 1915–16 Über Hyperphalangie des Daumens. Ant. Anz., Bd. 48, S. 565.

RECENT BOOKS

History and Bibliography of Anatomic Illustration, in its relation to anatomic science and the Graphic Arts, by Ludwig Choulant, translated and edited with notes and a biography by Mortimer Frank, B.S., M.D., 436 pages, illustrated, The University of Chicago Press, Chicago, 1920.

A Laboratory Manual and Text-Book of Embryology, by Charles W. Prentiss, Late Professor of Microscopic Anatomy, Northwestern University Medical School, Chicago. Revised and rewritten by Leslie B. Arey, Professor of Microscopic Anatomy; Third edition, enlarged, octavo, 412 pages, 388 illustrations, many in color. Philadelphia and London, W. B. Saunders Company, 1920. Cloth, $5.50.

The Form and Functions of the Central Nervous System, an introduction to the study of nervous diseases, by Frederick Tilney, M.D., Ph.D., and Henry Alsop Riley, A.M., M.D. Foreword by George S. Huntington, Sc.D., M.D.; 1020 pages, 763 illustrations of which 56 are in colors; New York, Paul B. Hoeber, 1921. $12.00.

The Growth and Shedding of the Antler of the Deer; the histological phenomena and their relation to the growth of bone, by William Macewen, F.R.S., illustrated, 110 pages, Glasgow, Maclehose, Jackson & Co., 1920, 10 s. 6 p.

The Psychology of Functional Neuroses, by. H. L. Hollingsworth, Associate Professor of Psychology, Columbia University, 260 pages, D. Appleton and Company, New York, 1920.

On Boneformation, its relation to tension and pressure, by Dr. Murk Jansen, Illustrated, 114 pages, Longmans, Green and Co., London and New York, 1920. $7.50.

The Fundamentals of Human Anatomy, including its borderland districts from the view point of a practitioner, by Marsh Pitzman, A.B., M.D., 101 illustrations, 356 pages, St. Louis, C. V. Mosby Company, 1920. $4.00.

An Introduction to the Study of Cytology, by L. Doncaster, Sc.D., F.R.S., Cambridge University Press, Illustrated, 280 pages, Cambridge, England (G.P. Putnam's Sons, 2 West 45th Street, New York). 1920.

Resumen por la autora, Helen Dean King.
The Wistar Institute of Anatomy and Biology.

Un estudio comparativo de la mortalidad durante el nacimiento en la rata albina y en el hombre.

Los datos reunidos durante un periodo de cinco años demuestran que en un total de 31,670 ratas albinas recién nacidas, 415, o sea el 1.3 por ciento, nacieron muertas. Concediendo que hay probablemente error en estos cálculos, la mortalidad normal durante el nacimiento en la rata albina es de unos 2 por ciento, esto es la mitad de la misma mortalidad en el hombre. En ambas especies la relacion normal de los individuos nacidos con vida es próximamente de unos 105.5 machos por cada 100 hembras; en los nacidos muertos esta relación aumenta hasta ser de 130 machos por cada 100 hembras. La mortalidad postnatal es un poco mayor que la mortalidad durante el nacimiento tanto en la rata como en el hombre.

Las causas a que debe atribuirse cerca de la mitad de los nacimientos de individuos muertos en el hombre, esto es las enfermedades infecciosas, implantación defectuosa y obstrucción mecánica durante el nacimiento, al parecer no influyen sobre la mortalidad natal de la rata. Las causas principales de tal mortalidad en dicho animal parecen ser los factores que afectan la nutrición del feto, tales como la edad y condición física de la madre, lactancia y número de individuos de la cría. Tanto en la rata como en el hombre, el feto macho parece ser intrínsecamente más débil que el hembra, y por consiguiente puede ser influído adversamente con más facilidad por las condiciones inímicas al desarrollo normal. La autora propone una hipótesis que supone que la diferencia en el vigor constitucional de ambos sexos depende de la estructura diferente de la cromatina en el zigoto macho y en el hembra.

Translation by José F. Nonidez
Cornell Medical College, New York

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, DECEMBER 27

A COMPARATIVE STUDY OF THE BIRTH MORTALITY IN THE ALBINO RAT AND IN MAN

HELEN DEAN KING

The Wistar Institute of Anatomy and Biology

Literature dealing with birth statistics for man contains numerous references both to the number and to the sex of the stillborn; but in none of this literature, nor in any of the many works covering various phases of animal breeding, is there any detailed information regarding birth mortality in other mammals. It has seemed worth while, therefore, to record the data for stillbirths in the albino rat that have been collected in the course of an extensive series of breeding experiments carried on in the animal colony of The Wistar Institute of Anatomy and Biology. Life processes in the rat accord in many ways with those for man, as Donaldson ('06, '08, '18) has pointed out, and the data given in the present paper indicate that there is also a close agreement in their normal sex ratio, in their birth mortality, and in the sex proportions of the stillborn.

The collection of data regarding the birth mortality in the albino rat was begun June 1, 1913, and carried on uninterruptedly for four years; it was resumed the beginning of June, 1918, and discontinued June 1, 1919. In any investigation of this character it is essential that all of the individuals in a given litter should be recorded, if the data are to have much statistical value. This necessitates, in the case of the rat, an examination of the litters at birth, or shortly after, since stillborn young left for a longer time in the nest are sometimes destroyed. While it is known that the data for the great majority of litters included in this study are complete, there is a probability that some of the stillborn young were omitted from the records, since all litters could not be examined at or close to the time of birth. The magnitude of the probable error is not great enough, however, to affect

the general conclusions that have been drawn, though more exact data might change somewhat the various percentages given.

Normally, young rats begin suckling soon after their birth, and as at this time and for some days afterward the skin over the abdomen is semi-transparent, any milk in the intestinal tract is readily seen and is a sure indication that the individual was alive when born. There is, therefore, no difficulty in distinguishing the stillborn individuals from those that died later, even though the litter is not examined until a day or two after it is cast. Throughout this paper the word 'stillborn' is applied only to young rats that lived through the normal gestation period (twenty-one to twenty-three days) and died shortly before or during birth, and the mortality data given are for such individuals only. Only a very few cases of abortion have been found in the course of breeding experiments with the rat extending over a period of eleven years and comprising many thousands of individuals; none of these are included in the present study.

THE NORMAL PERCENTAGE OF STILLBIRTHS

During the period in which the birth mortality statistics for the albino rat were being collected, a total of 253 litters were found in which one or more individuals were stillborn. Data for these litters, arranged according to the year in which the records were taken, are given in table 1.

The data given in table 1 show that there was considerable variation in the number of stillbirths occurring in different years. Such variation was to be expected, since the total litter production in the colony varied greatly from year to year (table 2). The percentage of stillbirths in the total number of individuals involved, however, is remarkably constant for all sets of data, as the range of variation is from 20 to 26.2 per cent only (table 1). Since in each year that records were taken at least one-fifth of the young in a considerable number of litters were dead at birth, it is evident that the mortality was not due to chance, but to some disturbance in the metabolism of the mother that tended to involve the litter as a whole.

In order to show the normal percentage of stillbirths in the entire colony, data for the total litter production during the period that the mortality records were taken are given in table 2.

TABLE 1

Data for living and for stillborn young in 253 litters of albino rats. Groups arranged according to the year in which the records were taken

YEAR	NUMBER OF LITTERS	TOTAL NUMBER OF YOUNG	LIVING YOUNG			STILLBORN YOUNG			
			Males	Females	Number of males to 100 females.	Males	Females	Number of males to 100 females	Per cent stillborn in total number of young
1913–1914	29	225	81	99	81.8	25	20	125.0	20.0
1914–1915	78	570	222	225	98.7	70	53	132.1	21.6
1915–1916	35	289	121	104	116.3	36	28	128.6	22.1
1916–1917	51	339	135	115	117.4	48	41	117.1	26.2
1918–1919	60	394	152	148	102.8	55	39	141.0	23.8
	253	1,817	711	691	102.9	234	181	129.3	22.8

TABLE 2

Showing the total number of individuals, including the stillborn, that were produced in a colony of albino rats during a period of five years

YEAR	TOTAL NUMBER OF LITTERS	TOTAL NUMBER OF INDIVIDUALS	MALES	FEMALES	NUMBER OF MALES TO 100 FEMALES	NUMBER OF STILLBORN	PER CENT STILLBORN
1913–1914	899	6,677	3,379	3,298	102.5	45	0.67
1914–1915	945	7,065	3,690	3,375	109.3	123	1.74
1915–1916	828	6,443	3,273	3,170	103.2	64	0.99
1916–1917	1,023	7,131	3,597	3,534	101.8	89	1.25
1918–1919	625	4,354	2,217	2,137	103.7	94	2.16
	4,320	31,670	16,156	15,514	104.1	415	1 31

As table 2 shows, from 600 to 1000 litters of albino rats were born each year during the period covered by the investigation. The percentage of stillbirths in the total litter production varied considerably in different years, and for the 31,670 births was 1.31 per cent. Assuming, for reasons to be given later, that at most only 8 per cent of the stillbirths that occurred were not recorded, it would appear that the normal birth mortality in the colony, under existing conditions of environment and of nutrition, was not greater than 2 per cent.

During the past seventy-five years an extensive literature has appeared dealing with the birth statistics for man in various countries of the world. From the data given in this literature, Nichols ('07) has compiled a table showing the living and the stillbirths throughout the world during the period from 1751 to 1903. In the enormous total of 447,019,379 births recorded, 13,635,986, or 3.04 per cent, were stillbirths. From a statistical standpoint the various sets of data used by Nichols are not of uniform value, since the laws regarding the registration of births vary greatly not only in different countries, but in different sections of the same country (as in the United States), and therefore many of the records are known to be incomplete. The records of stillbirths, especially, are very faulty, partly because in many countries their registration is not required and partly because the data obtained include fetuses aborted at various stages of gestation. With reference to human births, it may be noted, the word 'stillborn' is used to designate fetuses that are at least seven months of age when born; fetuses aborted at earlier stage of development are not ordinarily included in birth statistics.

More recent series of statistics show a birth mortality for man varying but slightly from that given by Nichols. Thus Auerbach ('12) states that in over 100,000 births as registered in Budapest, 3.3 per cent were stillbirths, and Terry ('17) has shown that in a total of 449,744 births recorded in Massachusetts during 1910 to 1914 there were 15,911, or 3.2 per cent, of stillbirths.

In certain countries in which the laws regarding the registration of births are relatively strict, the percentage of stillbirths is somewhat higher than that given by Nichols. For example, statistics for Prussia during the period from 1872 to 1881 show, according to Düsing ('84), that the stillbirths formed 4.67 per cent of the total of 10,577,478 births; birth statistics for the United States during 1918 show 3.56 per cent of stillbirths in a total of 1,412,283 births (Davis, '20).

The data for human births in one year in selected cities of the United States, as collected by The Children's Bureau of the U. S. Department of Labor, form a unique and valuable series (Duke, '15; Duncan and Duke, '17; Allen, '19; Dempsey, '19). Although

the number of births recorded is very small when contrasted with the large numbers given above, the great care taken to make these records as complete as possible gives to the data great statistical value.

Birth statistics for four selected cities, as collected by The Children's Bureau, are given in table 3.

TABLE 3

Summary of human births in one year based on data collected by the U. S. Children's Bureau in selected cities of the United States. 1, Brockton, Mass.; 2, Johnstown, Pa.; 3, Manchester, N. H.; 4, Saginaw, Mich.[1]

	TOTAL NUMBER OF BIRTHS	NUMBER OF MALES TO 100 FEMALES	LIVING YOUNG				STILLBORN YOUNG				
			Number of individuals	♂	♀	Number of males to 100 females	Number of individuals	♂	♀	Number of males to 100 females	Per cent stillborn in total number of young
1	1,247	105.77	1,210	623	587	106.13	37	18	19	94.73	2.96
2	1,551	110.16	1,463	761	702	108.40	88	52	36	144.44	5.67
3	1,643	101.10	1,564	781	783	99.74	79	45	34	132.35	4.80
4	1,015	107.99	981	507	474	106.92	34	20	14	142.85	3.34
	5,456	105.96	5,218	2,672	2,546	104.94	238	135	103	131.06	4.36

[1] Statistics of births during one year in a fifth city, Waterbury, Conn., have also been published by the Children's Bureau (Hunter, '18). The data given show a total of 2,654 births of which 3.2 per cent were stillbirths. Since sex data are given for only 53 of the 86 stillborn young, this series of data is excluded from table 3.

As shown in table 3, the percentage of stillbirths in the various cities concerned varied from 2.96 (Brocton) to 5.67 (Johnstown), and in the total of 5,456 births recorded there were 238, or 4.36 per cent, of stillbirths. In this series, therefore, the birth mortality is considerably higher than that in any series of data previously cited, yet in each of the papers in which the birth statistics are given it is stated that the number of stillbirths recorded is probably too low, owing to the great difficulty experienced in obtaining accurate information regarding such births. It has been estimated that at least 5 per cent of stillbirths are never recorded, even in localities in which the laws regarding their registration are most rigidly enforced.

Although the birth mortality in domestic animals would seem to be a matter of considerable importance to the stock breeder, there are only a few scattered references to it in works dealing with various phases of stock breeding, and practically no data having statistical value are available for analysis. According to Bernoulli ('41), records for Europe covering a period of ten years show that from 10 to 15 per cent of calves were dead at birth. As, however, a very great proportion of these deaths were undoubtedly abortions due to infectious disease, the normal birth mortality among full-term fetuses is yet to be determined.

Fairly complete records regarding living foals have been kept in various studs throughout Europe for many years, but data for the stillborn are very meager. Records compiled by Hoffman ('85) show that in a total of 1,556 foals, 87, or 5.6 per cent, were stillborn: Goehlert ('84), quoting Baumeister, states that on the average 6 per cent of all foals are born dead: 4 per cent of these are cases of abortion and 2 per cent are of foals at the end of term. In neither of these papers are any data given that show the sex proportions among the stillborn young.

Available evidence thus seems to indicate that in the higher mammals from 2 to 4 per cent of full-term fetuses are dead at birth, and it is probable that at least half of this mortality is due either to disease or to mechanical injury at birth.

THE SEX RATIO IN STILLBORN YOUNG

It is a matter of considerable interest whether the sex ratio, i.e., the number of males to each 100 females, in the stillborn is the same as that in the living young. For if there is a pronounced and constant difference between these two ratios, there must be some disparity between the sexes that is an important factor in the birth mortality.

In order to make possible a comparison between the sex ratio for the living and that for the stillborn young of the albino rat, it is necessary to ascertain the sex ratio that is normal for the species. Cuénot ('99) gives 105.6 males to 100 females as the sex ratio in thirty litters of albino rats; records for over 1000 litters of stock Albinos, as collected by King and Stotsenburg ('15),

show a sex ratio of 107.5 males to 100 females; while the data as given in table 2 of the present paper indicate a sex ratio of 104.1 males to 100 females in a total of 31,670 births.

The normal sex ratio for any species can be obtained only by ascertaining the sex proportions among all of the offspring of a considerable number of females kept under favorable conditions of environment and of nutrition during the entire period of their reproductive activity. None of the sex ratios for the albino rat as given above can, therefore, properly be taken as the norm, since none of them are based on completes series of data. Records covering the complete breeding history of a number of stock albino females have recently been obtained, however, and they show that the sex ratio in the newborn, including those dead at birth, is about 107 males to 100 females. This ratio, therefore, is the one that will be taken as the norm for comparison with the sex ratio in the stillborn.

On referring to table 1 it is found that in each year that mortality data for the albino rat were recorded there was a very great excess of males among the stillborn. While the number of stillbirths in any year was relatively small, the fact that in each set of records the sex ratio varies from the norm in the same direction and to a very considerable degree adds materially to the value of the data. In the total of 415 stillbirths recorded the sex ratio was 129.3 males to 100 females. This ratio is 26 points above that for the living young in the litters concerned, and as it is 22 points higher than the sex ratio taken as the norm (107 males to 100 females) the deviation is much too great to be considered as within the limits of normal variation. Granting that the records for stillbirths are incomplete, there is no reason to suppose that the sex ratio in the unrecorded stillborn would differ materially from that for the recorded stillborn as given in table 1. The evidence at hand, therefore, indicates that in the rat the mortality at birth is far greater among the male than among the female young.

According to Rauber ('00), as early as the year 1660 Graunt showed that more boys than girls were born in the city of London, and this finding has been confirmed by practically every collector

of human birth statistics since that time. Nichols' very comprehensive table of birth statistics, to which reference has already been made, shows a sex ratio of 105.5 males to 100 females in over four hundred million living births. More recent data give practically this same ratio: thus data for 171,297 living births of white and colored children in Cuba during the period from 1904 to 1906, as given by Heape ('09), show a ratio of 105.46 males to 100 females; while among the 465,655 births in Massachusetts from 1910 to 1914 the sex ratio is 105.41 males to 100 females (Terry, '17). Statistical evidence from many different sources thus seems to warrant the conclusion that in all civilized countries of the world there is an excess of males among the living young; the ratio which may be considered as the norm being about 105.5 males to 100 females. This ratio, as several investigators have pointed out, is remarkably constant and is maintained "through periods of war and of peace, of famine and of plenty, and under a great variety of racial and of climatic conditions; the variations, as a rule, being not greater than one per cent" (Pike, '07).

Available statistics for the sex of stillborn children are admittedly very incomplete, yet millions of such births have been recorded and they invariably show a fairly uniform sex ratio that differs in a marked degree from the sex ratio which is the norm for the living young. A few series of investigations may be cited to indicate the trend of such statistics in general. In the 13,635,986 stillbirths compiled by Nichols there were 131.6 males to each 100 females, the range of variation in the number of males being from 130 to 140 in the great majority of cases. In the records for stillbirths in various countries of Europe, as tabulated by Lewis and Lewis ('06), the number of males to 100 females varies from 120 to 170, with the average around 130; Heape's ('09) data for Cuban births shows a sex ratio of 144.45 males to 100 females among the stillborn, while Hirsch ('13) gives 127.9 as the number of males to 100 females in the stillborn young recorded in Germany during 1908 to 1909; and, finally, the birth statistics of the United States for the year 1918 indicate a sex ratio of 137.1 males to 100 females among the stillborn (Davis, '20).

All of the various series of records given above show that the sex ratio in the stillborn is much higher than that in the living young, and sex statistics for aborted fetuses indicate that the excess of boys becomes greater the earlier the month of pregnancy in which the fetus dies (Rauber, '00; Nichols, '07; Auerbach, '12; Carvallo, '12). This latter fact is of great importance, since it indicates that one, at least, of the chief causes for the excessive mortality among males at birth must be sought in conditions that exist in early rather than in late stages of gestation.

In 1841 Bernoulli called attention to the fact that the sex ratio at birth is not the ratio in which the young are conceived, and he concluded that the true sex ratio for man is about 108.2 males to 100 females. This ratio is practically the same as the 'primary' sex ratio recently calculated by Jendrassik ('11) and by Schultz ('18). The fact that in man the sexes are very evidently not conceived in equal numbers is a decided stumbling-block in the way of any theory of sex determination that postulates chance as the chief factor in deciding whether a given ovum shall become male or female. Morgan ('19) has recently offered the following explanation for the constant sex ratio in man:

> Since male babies die oftener than females, the difference has been said to be an 'adaptation,' with the implication that it calls for no further explanation. Several possible solutions suggest themselves. The male-producing sperm bearing the sex-chromosome may more frequently develop abnormally than the female-producing sperm. Again, since the spermatozoa must, by their own activity, travel the entire length of the oviduct to reach the egg as it enters the tube, the greater size or weight of the female-producing sperm may give a slight advantage to the male-producing sperm in the long trip up the tube. This would lead to an excess of males.

Since there is no evidence at present that one kind of spermatozoa is more active or more inclined to be abnormal than the other, it must be admitted that the above explanation for the male excess in human offspring is not an entirely satisfactory one.

A comparison of the sex ratios found in the newborn of the rat with the corresponding ones for man show that they agree closely in all cases. The sex ratio that is normal for the living young at birth is practically the same in both species, being about 105.5

males to 100 females; in both species, also, the sex ratio in the stillborn is much higher than that in the living young, averaging about 130 males to 100 females. This striking similarity in the sex ratios of two such widely separated mammals as the rat and man is a matter of considerable theoretical interest, and it may have a practical bearing as well, since through carefully controlled experiments on the lower form it may be possible to obtain information that will help to check the appalling birth mortality among human offspring.

Although a considerable body of statistics has been collected by Düsing ('84) and by Wilckens ('86), among others, regarding the normal sex ratio in domestic animals, practically no information is available concerning the sex proportions in the stillborn. In fact, the only reference to this subject that I have been able to find is in a paper by Goehlert ('82) which deals chiefly with the inheritance of coat color in the horse. Goehlert states that in 135,826 living foals born in various studs throughout Europe the sex ratio was 96.57 males to 100 females. Then follows this significant statement: "Derselbe steigert sich bei den todtgeborenen auf 106 bis 107 Hengst gegen 100 Stutenfohlen." The data on which the above statement is based are not given, but if they are extensive and accurate enough to have statistical value, they indicate that the sex ratio in stillborn foals is some 10 points higher than that in living foals. Thus in man, in the rat, and in the horse, the only mammals for which data are at present available, the birth mortality is apparently far greater among the male than among the female young. It is not improbable that future investigations will show that this condition is characteristic of the Mammalia generally.

SEASONAL VARIATIONS IN THE PERCENTAGE OF STILLBIRTHS

It has been claimed by Düsing ('84) that seasonal variations in temperature, through their action on nutritive conditions, affect not only the sex of developing fetuses, but the percentage of stillbirths as well.

The desirability of recording data for stillborn rats according to the month of the year in which birth occurred was not realized

when this investigation was begun, consequently only the data collected during the last year can be grouped by seasons as shown in table 4.

Stillbirths were recorded in the colony during every month of the year, the smallest number (3) being found in May, the largest (14) in September. On grouping the data as shown in table 4, it is seen that the 94 stillbirths were very evenly distributed throughout the different seasons, the variation in number being from 21 (spring) to 25 (autumn). The percentage of stillbirths in the total litter production, however, shows a wide range of variation in different seasons, being nearly twice as great in

TABLE 4

Showing the percentage of stillbirths in the albino rat colony from June 1, 1918, to June 1, 1919. Data arranged according to the season of the year in which birth occurred

SEASON	TOTAL NUMBER OF LITTERS	TOTAL NUMBER OF YOUNG	NUMBER OF STILLBIRTHS	PERCENTAGE STILLBIRTHS IN TOTAL NUMBER OF YOUNG
Spring (March to May)	183	1,349	24	1.78
Summer (June to August)	166	1,134	21	1.85
Autumn (September to November)	120	821	25	3.04
Winter (December to February)	156	1,050	24	2.28
	625	4,354	94	2.16

autumn (3.04) as in summer (1.78). The data given in table 4 are, of course, too few to have much statistical value, but they seem to indicate that the percentage of stillbirths tends to vary somewhat with the season, reaching its highest point in the autumn months. Lacking adequate means of heat regulation, rats suffer severely from high temperature, and the young born late in summer and in the autumn are, as a rule, inferior to those born at other seasons as regards their power of growth, resistance to disease, fertility, and longevity. It is not surprising, therefore, to find that this lowering of the physical tone of the animals at a definite season of the year is followed by an increase in the birth mortality.

From an analysis of the data for over ten million births occurring in Prussia from 1872 to 1881, Düsing ('84) concludes that "bei den Kindern, welche im Anfang des Jahres erzeugt und im Herbst geboren werden, zeigen sich die wenigsten (3.6 per cent), dagegen bei denen, welche im Frühjahr gezeugt und in Winter geboren werden, die meisten tot-geburten (4.4 per cent)." Other groups of statistics for human births do not support Düsing's conclusions, however, Thus, data compiled by Davis ('20) show that in the birth registration area of the United States during 1918 the lowest percentage of stillbirths occurred during the summer (3.07 per cent), and the highest in the autumn (3.79 per cent); while birth statistics for the city of Philadelphia covering a period of ten years (Sozinskey, '85) and also those for Boston during 1891 to 1910 (Whipple, '19) indicate no appreciable variation in the rate of stillbirths during different seasons of the year.

From available evidence it would appear that the birth mortality in man is but little influenced by the season of the year in which either conception or birth occurs. Since man has a highly developed mechanism for heat regulation, moderate changes of temperature have very little effect on body metabolism and therefore cannot, under ordinary circumstances, influence the nutrition of the fetus, as Düsing claims.

POSTNATAL MORTALITY

Since deaths that occur among the young within a few days after birth are traceable, in many cases, to prenatal causes that are responsible for a certain proportion of stillbirths, a brief consideration of postnatal mortality is included in the present paper.

Little exact information is available, as yet, regarding the mortality among young rats during the week after birth. The number of such deaths in any large colony is considerable, but what proportion of them is due to prenatal causes cannot be determined, since a great part of such mortality is always due to causes that are purely accidental, such as smothering of the young by the crowding of adults into the nest when they are cold or

frightened and death from exposure or starvation when the young leave the nest and are not carried back by the mother.

Records kept from June 1, 1918, to June 1, 1919, show that ninety-eight rats died within three days after birth from causes that were undetermined. As during this year 4,250 living young were born in the colony, the postnatal mortality was 2.3 per cent, or slightly greater than the birth mortality during the same period (table 2), Although the great majority of these deaths were undoubtedly accidental, some of them were unquestionably due to prenatal causes that so affected the constitutional vigor of the individual that death was inevitable. Occasionally litters are cast in which one or more of the members are very much under normal size. These small individuals are the so-called 'runts,' which are frequently found among multiparous mammals, and since they are usually unable to compete with the larger and more vigorous individuals of the litter in their efforts to obtain food, they generally die within a few days after birth. Under very favorable conditions some of these undersized individuals are able to survive and to reach maturity, but they never attain the size of the normal members of the litter and they are usually sterile (King, '16). Since runts are found most frequently in very large litters cast by young females and in litters cast by females that are not in good physical condition, they are evidently individuals with relatively low initial vitality that were subjected to conditions inimical to growth during the intrauterine period; the weaker among them die soon after birth, those that survive are among the physically unfit that generally 'drop out' at a relatively early age.

Among the 98 young recorded as dying shortly after birth there were 42 males and 56 females, or a sex ratio of 75 males to 100 females as contrasted with a sex ratio of 141 males to 100 females in the stillborn young found during the same period (table 1). The marked difference between these two ratios is readily explicable. Factors responsible for the great excess of males among the stillborn can act only to a very limited extent in influencing the sex ratio in the young that die after birth, and postnatal mortality due chiefly to accidental causes might be ex-

pected to take a heavier toll from the females, since at birth the females are somewhat smaller, as a rule, than the males (Donaldson, '06; Jackson, '13; King, '15).

The question of postnatal mortality among human offspring involves so many different factors that an adequate consideration of the subject cannot be attempted here. The many investigations that have been made show that the mortality is very high during the first month after birth, averaging about 5 per cent of all young. About one-fourth of these deaths, it has been estimated, are due to improper care or to disease, the remaining can be attributed to premature birth, injuries at birth, or to congenital debility (Ashby, '15; Hunter, '18; Eastman, '19; Dempsey, '19).

Sex statistics for infants dying under one year of age, as collected by a number of investigators in various countries (Düsing, '84; Rauber, '00; Prinzing, '06; Nichols, '07; Dutton, '10; Pinard et Magnan, '13; Kroon, '17; Ashby, '15; Davis, '18, '19, etc.), all show that infant mortality is considerably greater among boys than among girls, and that, while it varies considerably in different localities and under different conditions, on the average about 120 boys die to each 100 girls.

A comparison of the findings for the rat with those for man shows that in both forms the postnatal mortality is somewhat higher than the birth mortality; in the rat this mortality is chiefly due to accidental causes that seemingly tend to kill more females than males, while in man infant mortality is traceable in many cases to 'congenital debility' which is apparently far more fatal to males than to females.

CAUSES OF BIRTH MORTALITY

Barring accidents, there are six leading causes to one or another of which practically all stillbirths in mammals can be ascribed: 1) malposition of the fetus leading to abnormal development; 2) infectious disease; 3) mechanical obstruction to birth, including size of the fetus; 4) physical condition of the mother; 5) age of the mother; 6) congenital debility. The part played by these various factors in the birth mortality in the rat and in man will be discussed briefly in the following sections.

a. Malposition of the fetus and disease as causes of birth mortality

Faulty implantation is responsible for the abnormal development of many ova in the rat (Huber, '15), but these ova, as a rule, die at an early stage and are absorbed in situ. Little is known, as yet, regarding the death in utero of older embryos. The examination of a number of gravid females indicates that this phenomenon is not as common in the rat as it is in many other multiparous mammals (Stahl and Henneberg, '02; Hammond, '14). So-called 'monsters,' which arise through faulty implantation and consequent inadequate nutrition of the embryo, comprise about 1 per cent of all human fetuses at birth (Mall, '08), but they are very rare among newborn rats. In the course of an examination of over 50,000 young rats I have found but four such fetuses, and in all of these the body appeared perfectly normal, but the head was hydrocephalic.

Infectious diseases are responsible for an appalling number of deaths among human offspring and among the young of cattle, but no cases are known, as yet, in which stillbirths in the rat could be ascribed to this cause. Neither the rat scourge, so-called 'pneumonia,' nor other diseases common to the rat are transmitted to the fetus as far as is known. From the evidence at hand, therefore, infectious disease can be eliminated as a cause of stillbirth in the rat, though illness of the mother, as will be shown later, is a potent factor in birth mortality.

b. The size of the fetus as a cause of birth mortality

Since the size of the fetus is an important factor in human birth mortality (Düsing '84; Nichols, '07; Dutton, '10; Hirsch, '13), it is conceivable that this factor may also play a rôle in the birth mortality in the rat. The following series of observations was made to determine this point.

Fifty-nine litters of rats, in which one or more members were stillborn, were obtained at birth. Each of the young rats was taken from the mother as soon as it was cast, the placenta was removed, and the body weight taken immediately. Data regarding the age and general physical condition of the mother at

the time of parturition were also recorded, since these factors had to be taken into account as possible causes of birth mortality.

The body weight records for the 306 living and for the 137 stillborn young in these fifty-nine litters are shown in table 5. For purposes of later analysis the data are arranged in three groups according to the physical condition of the mother at the time of parturition.

TABLE 5

Sex and body-weight data for living and for stillborn young in fifty-nine litters of albino rats. Groups arranged according to the physical condition of the mother at the time of parturition

GROUP	PHYSICAL CONDITION OF MOTHER	LIVING YOUNG						STILLBORN YOUNG						Percentage stillborn in total number of young
		Number of individuals		Average body weight all individuals		Average body weight largest individuals		Number of individuals		Average body weight all individuals		Average body weight largest individuals		
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	
				gms.	*gms.*	*gms.*	*gms.*			*gms.*	*gms.*	*gms.*	*gms.*	
1	Good	68	51	4.57	4.28	4.91	4.52	21	21	4.20	3.75	4.50	4.11	26.1
2	Poor	43	60	4.24	4.17	4.45	4.42	46	28	4.15	4.08	4.40	4.31	41.8
3	Good, but female young	43	41	4.12	3.84	4.53	4.40	12	9	3.56	3.80	3.85	3.85	20.0
		154	152	4.41	4.13	4.66	4.39	79	58	4.11	3.81	4.36	3.99	30.9

In table 5 the final averages, computed from individual not from grouped data, confirm the findings of Donaldson ('06), of Jackson ('13), and of King ('15), that in the rat the living male is heavier at birth than the living female. They show, also, that this same relation is found among the stillborn; the difference between the body weights of the two sexes averaging about 0.30 gram in each case.

In each of the three groups given in table 5, the living young, both males and females, have a heavier birth weight than the stillborn young, the final averages indicating a difference of 0.30 gram for each sex. Obviously the body-weight relations between the living and the stillborn young would be just the reverse of that shown above if the size of the individual is a determining cause of birth mortality. When the average body weights for

the largest of the living and the largest of the stillborn in each group are compared, the result shows conclusively that the size of the fetus is not a cause of death at birth, since in all three groups the average body weight of the largest living individual of both sexes exceeds that of the largest stillborn individual in the corresponding group. In this instance, also, the final averages show a difference of 0.30 gram in favor of the living young.

In man multiple births are the exception, not the rule as in the rat, so in this respect conditions in these two species are radically different. It is not so much the weight of the fetus as the size of the head that is responsible for the death of many infants, particularly boys. The deaths from this cause and those due to other forms of mechanical obstruction to birth comprise about 10 per cent of all human stillbirths, according to various observers.

The data given in table 5 show that the stillbirths formed 30.9 per cent of the total of 443 births in fifty-nine litters of albino rats. In collecting these data there was no error, since the litters were obtained at the time of birth and all of the young recorded. In the total of 253 litters containing stillborn young that were obtained during a period of five years, the birth mortality among 1817 individuals, as registered, was 22.8 per cent (table 1). The difference between these two sets of data would seem to indicate that at most 8 per cent of the stillbirths in the colony were not recorded. This error is not sufficiently large to invalidate any of the conclusions drawn from the records as they stand.

c. The physical condition of the mother as a cause of birth mortality

It requires but little experience in the handling of albino rats to determine from the general appearance of an animal whether or not it is in good physical condition. Alert animals of large size, having clear eyes and thick, glossy hair, are usually in excellent condition and free from disease. On the other hand, labored breathing, rough hair, dark red eyes, sluggish movements, and relatively light body weight are all evidences of poor physical condition and generally indicate that the animal is in an advanced stage of 'pneumonia.'

Table 6 shows the age, body weight, and general physical condition of the fifty-nine albino females that gave birth to the young whose body-weight data are given in table 5.

The first group in table 6 comprises twenty females that were apparently in good physical condition at the time of parturition, as was indicated not only by their general appearance and behavior, but also by the fact that they weighed, on the average, over 15 grams more than the 'standard' body weight for breeding females of the same age (Donaldson, '15). The average body weights of their young at birth exceeded those of the young cast by females in poor condition (cf. group 1 and group 2; table 5), thus adding more evidence that "rats in good physical

TABLE 6

Data regarding the age, body weight, and general physical condition of the fifty-nine female albino rats that cast the litters recorded in table 5

GROUP	GENERAL PHYSICAL CONDITION OF FEMALES	NUMBER OF FEMALES	AVERAGE AGE OF FEMALES AT PARTURITION	AVERAGE BODY WEIGHT OF FEMALES AT PARTURITION	STANDARD BODY WEIGHT FOR AVERAGE AGE OF FEMALES AT PARTURITION (DONALDSON, '15)
			days	*gms.*	*gms.*
1	Good	20	202	224.4	209.1
2	Poor	26	244	190.7	220.0
3	Good; but female very young	13	99	143.7	146.2

condition bear young with a birth weight considerably above that of the young cast by females in poor condition" (King, '15). Each female in this group gave birth to a litter that contained, on the average, two stillborn to six living young (table 5). Since these stillbirths cannot be ascribed either to mechanical obstruction to birth, to abnormal development, nor to infectious disease, it would appear that they must have been caused by some other prenatal condition that adversely affected the vitality of the young.

Examining the history of the mothers, as kept on record cards, it was found that five of the females in this group were nursing young at the time that the litter containing stillborn young was

cast; eight of the females gave birth to very large litters containing ten or more members; three females were over fifteen months of age at the time of parturition. No reason can be assigned for the presence of stillborn young among the offspring of the remaining four females in this group. It is possible, perhaps, that these females were in early stages of 'pneumonia' which had not as yet altered either their general appearance or their body weight, but had already affected their body metabolism in such a way as to adversely influence the development of the fetal young.

The second group in table 6 comprises twenty-six females that were obviously in bad physical condition at the time that their litters were cast. These females were, on the average, some 30 grams under the 'standard' body weight for breeding females of the same age, and the birth weights of their young were very low (table 5). It is of interest to note that the percentage of stillbirths in the total number of young cast by these females was relatively very high (cf. group 1 and group 2; table 5). The majority of the females in this group were obviously suffering from 'pneumonia;' three of them were in such an advanced stage of the disease that they had to be killed as soon as the litter was cast.

d. The age of the mother as a factor in birth mortality

It has already been shown that litter size in the albino rat is influenced to a considerable extent by the age of the mother (Slonaker, '12; King, '16 a), and it is possible that the viability of the young at birth may also be affected by this same factor.

As already noted, three of the females in the first group of table 6 were over fifteen months of age when casting a litter that contained stillborn young. These females appeared to be in good physical condition, yet their litters were very small, and seven of the eleven young cast were dead at birth. The third group of table 6 comprised thirteen females that had an average age of only ninety-nine days when casting their first litter. Although each of these females was seemingly in good health, twenty-one in the total of 105 young were stillborn (table 5).

Since none of the females in these two groups showed any evidence of disease at the time of parturition, it is probable that the age of the mother, and not incipient 'pneumonia,' was the chief factor responsible for the high birth mortality in their young. The age of eighteen months marks approximately the end of the reproductive activity of the albino female, and toward its close, as at its beginning, there seems to be a strong tendency for the females to cast fewer individuals in a litter and a relatively greater proportion of stillborn young. This phase of the subject will be discussed later.

In considering the causes responsible for stillbirths among human offspring, the age of the mother is a factor that is usually ignored or assigned a very minor rôle. Various series of reliable statistics, however, indicate that the birth mortality is relatively high in children of very young and of very old mothers, so evidently the age factor has greater influence in this respect than is generally assumed. The trend of statistical evidence on this point is shown by the following examples. Whipple's ('19) analysis of the birth statistics for the city of Boston during the period from 1891 to 1910 shows that: "The percentage of stillbirths arranged according to the age of the mother gave the very high percentage of 11.1 per cent for mothers under 20 years of age, 4 per cent for age group 20–24, 5.1 for 25–29 years, 4.4 for 30–39 years, and 3.3 for ages over 40."

Far more comprehensive data regarding the effect of the age of the mother on birth mortality among the young are given by Davis ('20) in his study of the births in the registration area of the United States during the year 1918. In a total of 1,372,329 births in which the age of the mother was ascertained, there were 46,122 stillbirths. The percentage of stillbirths was 3.9 for mothers under twenty years of age, 3.2 per cent for mothers from twenty to thirty-nine years, and 5.4 per cent for mothers over forty. This study indicates that the percentage of stillbirths is much higher in children born to mothers at the beginning and at the end of the reproductive period, and its findings are confirmed by the birth statistics gathered by The Children's Bureau (Duke, '15; Duncan and Duke, '17; Allen, '19; Dempsey, '19),

which have already been given in table 3 of the present paper. They are shown again in table 7, arranged according to the age of the mother at the time that the birth of her child occurred. It will be noted that table 3 gives a total of 5,456 births, while table 7 registers only 5,452 births. This discrepancy is due to the fact that in four cases the age of the mother was not ascertained.

Nearly 90 per cent of the births registered in table 7 were those to women between twenty and thirty-nine years of age, the remaining 10 per cent were evenly divided between women that were under twenty and over forty. As this is about the normal distribution of births relative to the age of the mothers, a comparison of the percentages of stillbirths as given seems permissible.

TABLE 7

Data for human births, collected by the U. S. Children's Bureau (table 3), arranged according to the age of the mother at the time of parturition

MOTHER'S AGE IN YEARS	TOTAL NUMBER OF BIRTHS	LIVING BIRTHS	STILLBIRTHS	PER CENT STILLBIRTHS IN TOTAL NUMBER OF BIRTHS
Under 20	282	263	19	6.95
20 to 39	4,882	4,683	199	4.07
40 and over	288	268	20	6.94
	5,452	5,214	238	4.36

Table 7 shows clearly that in this set of records, as in those given by Whipple and by Davis, the percentage of stillbirths is correlated with the age of the mother. In table 7 the stillbirths formed only 4.07 per cent of all births to women at the zenith of the child-bearing period, while they were increased to nearly 7 per cent in the births to women at the extremes of the reproductive period.

When the data in table 7 are arranged according to the order of the birth, as is shown in each of the papers in which the separate sets of data are given, it is found that the percentage of stillbirths is higher for the first births and for those after the sixth than for the intermediate births. A series of birth statistics arranged in this manner is, of necessity, an age series, and it is

more probable that the observed variations in the percentage of stillbirths depend on the age factor rather than on the number of the pregnancy.

It has been claimed that the high birth mortality in children of very young mothers is due chiefly to the mother's ignorance of the proper hygienic laws that should be observed by pregnant women. This explanation cannot be offered to account for the increase in the percentage of stillbirths among the children of women over forty, however, since births at this age are rarely those of the first pregnancy. It seems probable that both at the beginning and at the end of the reproductive period physiological conditions incident to age are responsible in great part for the high birth mortality among the children born at this time.

e. Congenital debility as a cause of birth mortality

Stillbirths among human offspring not traceable to a well-defined cause are generally ascribed to 'congenital debility,' this term being used to indicate a lack of sufficient vitality in the fetus to render postnatal existence possible. Various series of investigations show that a very considerable proportion of stillbirths are attributed to this cause. For example, in 201 cases of stillbirths at term studied by Brothers ('96), over 50 per cent were classed as due to 'congenital debility,' while Waldvogel's ('13) studies led to a similar conclusion.

In the sense in which the term 'congenital debility' is used above, practically all stillbirths in the rat might properly be grouped under this heading, since in all cases so far found impaired vitality of the fetal young was seemingly the direct cause of the birth mortality; the underlying cause is discussed in the following section.

GENERAL DISCUSSION

This study has shown that in two important respects the statistics for the birth mortality in the albino rat accord in a most striking manner with those for man: 1) the sex ratio in the living young at birth is practically the same in both forms (about 105.5

males to 100 females); 2) the great excess of males among human stillborn finds its parallel in the high sex ratio which characterizes the stillborn of the rat (129 males to 100 females). In one respect only the birth records for these two forms do not agree. The normal percentage of stillbirths in human offspring (4 to 5 per cent) is at least twice that in the albino rat (table 2). This difference, however, is readily explicable. Factors which are responsible for about one-half of all human stillbirths, i.e., mechanical obstruction to birth, accidents, faulty implantation, and infectious disease, ordinarily play little, if any, part in the birth mortality in the rat. If stillbirths due to these causes are eliminated, the birth mortality among human offspring falls to about 2 per cent, which is close to the percentage of stillbirths which is seemingly normal for the albino rat when large numbers of breeding animals are kept under fairly uniform conditions of environment and of nutrition. The stillbirths in man which thus seem comparable to those in the rat, are those that, in general, are attributed to 'congenital debility,' this term, as already stated, being used to indicate that the fetus possesses such a low state of vitality at the end of term that it is incapable of independent existence. From the evidence at hand the great proportion of stillbirths in the rat can be attributed to the same cause.

The question at once arises as to the cause of this impaired vitality in the fetus and whether it is possible to control it so that the percentage of stillbirths will be materially decreased. If we consider those cases in the albino rat in which the stillborn young were obtained at the time of birth and the age and physical condition of the mothers noted (tables 5 and 6), it is found that the great majority of them occurred in litters of females that were suffering from disease, chiefly 'pneumonia,' or in those of females at the extremes of the reproductive period, while in a few cases the litters were very large or were cast by females that were suckling young at the time of parturition. Extensive series of breeding experiments extending over a period of a dozen years and covering the birth of many thousands of rats lead me to believe that practically all stillbirths in this animal occur under

one or another of these conditions. The one factor which seemingly might have affected the vitality of the fetal young in all of these cases is malnutrition.

The nutrition of the fetal young is a very complex process, and in its broadest sense it includes the absorption and the assimilation of food by the mother as well as its transmission through the placenta to the young. Any factor or physiological condition that adversely affects the normal metabolic processes upon which any phase of embryonic nutrition depends therefore indirectly influences the development of the young and may impair their vitality to a greater or less extent.

Let us consider in some detail the effect of the various factors that are apparently responsible for stillbirths in the rat. The rat scourge, 'pneumonia,' is a wasting disease, and as such profoundly affects all of the normal life processes in an animal affected with it. The living young cast by females having this disease in an advanced stage are usually very small and emaciated at birth, and their vitality is at such a low state that they are difficult to rear even when suckled by a vigorous foster-mother. There can be no question but that the fetal young suffer throughout the entire course of their intra-uterine existence from malnutrition, since the illness of the mother must interfere with her power to assimilate food and to transmit it to her offspring. With such a handicap to their normal development, it is not surprising that a large proportion of the young are not able to survive at birth.

The suckling of young, particularly if the litter is above the norm (seven) in size, is as a rule a severe strain on the nutritive reserve of the mother, as is shown by the fact that she usually loses considerable weight during this period unless she is in excellent health and abundantly supplied with food. Not infrequently a lactating female is also carrying a second litter. If one or both of these litters are large, the amount of food that the mother can assimilate and supply to her young, in addition to her own needs, is inadequate for the proper nutrition of all of the individuals concerned. The result is that the suckling young usually grow very slowly and show every evidence of being un-

derfed, and the gestation period of the fetal young is lengthened from one to several days, owing doubtless to the fact that the implantation of the fertilized ova is delayed (King, '13; Kirkham, '16). Here again malnutrition is obviously a factor that impairs the vitality of the fetal young and tends to increase the proportion of stillbirths. A similar explanation can be offered for the presence of stillborn young in litters of exceptionally large size. In these cases the inadequate nutrition of the young is indicated by the fact that all members of the litter are, as a rule, of small size and under normal weight at birth.

The fact that the proportion of stillborn young in litters cast by very young and by very old females is markedly greater than that in litters cast by females at the height of their reproductive activity indicates that the age of the mother is a factor of importance in birth mortality. Donaldson ('06) has shown that one year of a rat's life is equivalent to thirty years of human life. At the age of eighteen months, therefore, a female rat corresponds physiologically to a woman of forty-five years, and it can hardly be a coincidence that this age marks approximately the end of the reproductive activity in both species. The onset of puberty does not, however, correspond as closely in the two forms, since rats breed at three months of age. Age has a profound effect on all of the normal activities of the body, and it is not surprising, therefore, to find that the immaturity of the young mother and the physiological changes in the uterus incident to the approaching menopause seemingly inhibit the metabolic processes concerned with the nutrition of the fetal young. In such cases the young often suffer from impaired nutrition, and consequently the birth mortality among them is much greater than that among the offspring of females at the height of their reproductive activity.

It has been repeatedly demonstrated that a mature, well-developed albino female that is in good physical condition at the time of conception, will, if abundantly supplied with proper food during the entire gestation period, cast a litter containing only living, vigorous young that have relatively heavy birth weights. A special experiment recently made to test this point has given

rather astonishing results. The young cast by a mature female abundantly supplied with rich food during the gestation and lactating period were twice the normal weight at birth, and at thirty days of age, when weaned, they were over 200 per cent above the average weight of rat at this age. At sixty days of age the males in this litter had an average body weight of 260 grams, which is some 300 per cent above the 'standard' weight for males of this age. On the other hand, inadequate feeding of breeding females invariably leads to the production of small litters containing undersized individuals in which there is a high percentage of stillbirths. This fact was fully demonstrated in the early stages of an inbreeding experiment with these animals which has been carried on for some years in our colony (King, '18). Even when the food supply is ample, the present study has shown that in lactating females, in those carrying a very large litter, and in those suffering from disease or breeding at one extreme of the reproductive period, physiological conditions within the body of the mother may adversely influence the various processes upon which the nutrition of the young depends and thus lead in many cases to the death of a considerable proportion of the young at birth.

Conditions of human existence are so complex and so artificial in many cases that one would hardly venture to assert that the underlying cause of all cases of 'congenital debility' was malnutrition of the fetus due to the age or to some abnormal physical condition of the mother, yet the evidence at hand is strongly in favor of such a view. Inadequate nutrition of the young is certainly a potent factor in the birth mortality of the rat, and it probably plays an important rôle in the birth mortality of other mammals, including man. The maintenance of pregnant females under environmental and nutritive conditions favorable to the health of the mother and to the adequate nourishment of the fetal young throughout the entire gestation period, not merely near its close, would, therefore, undoubtedly lead to the birth of more vigorous young and to a marked decrease in the number of abortions and of stillbirths.

Whenever large groups of human birth statistics have been analyzed it has been found that the mortality among the males is very high, the sex ratio for the stillborn being at least 25 points higher than that in the living young. This same phenomenon appears also when a large series of birth data for the rat are examined (table 1), and it likewise is present in statistics for the horse, if Goehlert's ('82) records are reliable. Since the disparity between the sex ratios for the stillborn and those for the living young in these various groups of statistics is fairly constant and much too great to be considered as within the range of normal variation in the sex ratio, there must be some fundamental cause, founded on a difference in the constitution of the male and female organisms, that is responsible for the excessive mortality among the males at birth.

Düsing ('84) discusses this subject at considerable length, and he concludes that: "Die Knaben sterben also während Fötallebens häufiger als die Mädchen, weil viele derselben sich unter ungünstigen Ernährungsverhältnissen ausbilden, während sie, da sie durchschnittlich schwerer sind, sogar mehr Nahrung beanspruchen als die leichteren Mädchen." Since from the time that the sexes can be distinguished at about the sixth week of pregnancy the excess of males among aborted embryos is greater the earlier the age at which abortion occurs (Rauber, '00; Nichols '07; Auerbach, '12; Carvallo, '12), inadequate nutrition cannot well be considered as the primary cause of the greater mortality among male fetuses in general.

A most suggestive hypothesis that may have a very important bearing on this problem has recently been advanced by Lillie ('17) in his study of the action of sex hormones in producing the 'free-martin' in cattle: "It seems probable that the disturbance of the equilibrium that protects the male from the sex hormones of the mother would result in malformations of the male sex characters to a degree commensurate with the extent of the disturbance. There is, therefore, here a possible explanation for the greater mortality among male foetuses." Our knowledge of the action of sex hormones is as yet too meager to enable us to

advance a definite theory regarding the influence of these factors on fetal mortality.

Nichols ('07) states that three explanations are open to consideration to account for the heavy mortality among boys at birth "(a) the initial numerical preponderance of males; (b) the greater proportion of deaths of male fetuses occurring during parturition owing to their larger size; and (c) the intrinsically much greater mortality of males than of females in the earlier period of life, both antenatal and postnatal." Nichols assigns only a minor rôle to the first two of the causes enumerated above, and he concludes that:

> Obviously the main cause of the great preponderance of male stillbirths resolves itself into the question of the comparative mortality or death rate of the male and female sexes during the intrauterine period of existence. . . . . it is therefore obvious that the male constitution is intrinsically weaker, less hardy, and more susceptible to morbific and mortific influences, and has less vitality and resisting power against disease, than the female. The cause of this innate disparity of vitality between the two sexes we do not know; but the fact that it exists, that the antenatal mortality and death rate of males much exceeds that of female fetuses, accounts for the great excess of male over female stillbirths.

In the light of the recent researches in heredity it is conceivable that the inherent dissimilarity between the sexes as regards their constitutional vigor, which has been discussed in detail by Geddes and Thomson ('01), may have its basis in the germinal structure of the fertilized egg. From the work of Guyer (10) and of von Winiwarter ('12) on the spermatogenesis of man, and of Allen ('18) on the spermatogenesis of the rat, it is known that in both of these mammals the spermatozoa are dimorphic, one kind of spermatozoa having one more chromosome than the other; both kinds of spermatozoa are produced in equal numbers and both kinds, as far as known, are equally functional. The current theory of sex determination postulates that the spermatozoa containing the extra, or X-chromosome, are 'female-producing'; those lacking it are 'male-producing.' The fertilized ovum that is to develop into a female thus contains two X-chromosomes, while that having only the X-chromosome received from the mother

develops into a male. May not the difference in the constitutional vigor of the two sexes depend, in some way, upon the fact that the chromatin content of the female ovum is greater than than of the male ovum? One might suggest, perhaps, that the excess of chromatin brought into the egg by the 'female-producing' spermatozoan influences the ensuing interaction of the chromatin and the cytoplasm in such a way that the embryo becomes endowed with a constitution that is more stable and more vigorous than that of the embryo developing from an egg in which the initial amount of chromatin is less. Such an hypothesis is, of course, only tentative. Until our knowledge of heredity and of the sex-determining mechanism is greatly increased it will be futile to theorize as to the probable cause for particular characters associated with one sex or the other which seemingly do not depend upon the action of specific genes.

The great excess of males among aborted and stillborn fetuses is readily explained if we assume that, from the time of conception, the embryo that is to develop into a male has a constitution inherently weaker than that of the embryo that is to become a female. During the gestation period many factors, such as disease, unfavorable environmental conditions, physiological changes incident to age, etc., may lessen the mother's power of assimilating food and of transmitting it to her fetal young. Under these conditions, a male fetus possessing a relatively low initial vitality would be more severely handicapped by inadequate nutrition or by conditions unfavorable to normal development than would a female fetus having a greater initial vigor of constitution and more power to resist unfavorable environmental conditions. One, therefore, would expect to find the facts exactly as shown by various series of investigations cited in this paper, namely, that at all stages of gestation and at birth the mortality among the males is far greater than that among the females.

SUMMARY

1. Birth statistics collected during a period of five years show that in a total of 31,670 newborn albino rats 415, or 1.3 per cent, were stillborn. Allowing for the probable error in recording the

data, it would appear that under normal environmental conditions not more than 2 per cent of rat fetuses are dead at birth.

The most accurate statistics available indicate that the normal birth mortality in man is about 4 per cent. There are no data of value regarding the percentage of stillbirths in other mammals.

2. The normal sex ratio in newborn albino rats, including the stillborn, is about 107 males to 100 females; in man the sex ratio for the living young at birth averages about 105.5 males to 100 females, and if the stillborn are added the sex ratio rises to about 108 males to 100 females.

3. In each year that the mortality records for newborn rats were taken there was a pronounced excess of males among the stillborn, the sex ratio in the total number of such individuals being 129.3 males to 100 females (table 1).

Large series of birth statistics collected in many different countries show that the sex ratio among stillborn children is very high, averaging about 130 to 140 boys to 100 girls. The excess of boys becomes greater the earlier the month of pregnancy in which the fetus dies.

4. In the rat the percentage of stillbirths seems to vary somewhat with the seasons, being least in the spring and greatest in the autumn months when the breeding animals are suffering from the devitalizing effects of high temperature during the preceding summer (table 4). The birth mortality among human offspring does not, apparently, vary to any appreciable extent at different periods of the year.

5. Data collected during one year show that the mortality among young rats during the first three days after birth was 2.3 per cent, or slightly greater than the birth mortality in the colony during the same period (2.16 per cent); infant mortality in man during the first month after birth is about 5 per cent, or 1 per cent higher than the birth mortality.

Postnatal mortality in the rat is largely due to accidental causes which tend to kill more females than males; in man the death of many infants is traceable to prenatal causes which seemingly are more fatal to boys than to girls.

6. Factors responsible for a considerable proportion of the stillbirths among human offspring, such as, infectious disease, faulty implantation, mechanical obstructions to birth, including the size of the fetus, apparently play no part, ordinarily, in the birth mortality of the rat.

7. From the data obtained it appears that malnutrition is directly responsible for most of the stillbirths in the rat. Factors influencing the food supply of the fetal young, such as the physical condition and the age of the mother, the suckling of young, and the size of the litter carried, are therefore the chief causes of birth mortality in the rat.

8. Available evidence indicates that both in the rat and in man the male fetus is intrinsically weaker than the female, and therefore more susceptible to prenatal influences inimical to normal development. A tentative hypothesis is advanced that the difference in the constitutional vigor of the sexes has its basis in the different chromatin structure of the male and female zygote.

LITERATURE CITED

ALLEN, E. 1918 Studies in cell division in the albino rat (Mus norvegicus, var. alb.). III. Spermatogenesis: the origin of the first spermatocytes and the organization of the chromosomes, including the accessory. Jour. Morph., vol. 31.

ALLEN, N. F. 1919 Infant mortality. Results of a field study in Saginaw, Mich., based on births in one year. Children's Bureau, U. S. Dep't of Labor, Washington.

ASHBY, H. T. 1915 Infant mortality. Cambridge.

AUERBACH, E. 1912 Das wahre Geschlechtsverhältnis des Menschen. Arch. Rassen- u. Geschlechaftsbiol., Bd. 9.

BERNOULLI, C. 1841 Handbuches der Populationistik.

BROTHERS, A. 1896 Infantile mortality during child-birth and its prevention. Philadelphia.

CARVALLO, M. E. 1912 La masculinité dans les naissances humaines. Compte Rendu Assoc. Franc. pour d'Avancement des Sciences, T. 41.

CUÉNOT, L. 1899 Sur la determination du sexe chez les animaux. Bull. Sci. de la France et de la Belgique, T. 32.

DAVIS, W. H. 1918 Birth statistics for the birth registration area of the United States, 1916. Bureau of Census, Washington.

1919 Birth statistics for the birth registration area of the United States, 1917. Bureau of Census, Washington.

1920 Birth statistics for the birth registration area of the United States, 1918. Bureau of Census, Washington.

DEMPSEY, M. V. 1919. Infant mortality. Results of a field study in Brockton, Mass., based on births in one year. Children's Bureau, Dep't of Labor, Washington.

DONALDSON, H. H. 1906 A comparison of the white rat and man in respect to the growth of the entire body. Boas Anniversary Volume, New York.

1908 A comparison of the albino rat with man in respect to the growth of the brain and of the spinal cord. Jour. Comp. Neur., vol. 18.

1915 The rat: reference tables and data. Mem. Wistar Institute Anat. and Biology, no. 6.

1918 A comparison of the growth changes in the nervous system of the rat with corresponding changes in the nervous system of man. Proc. Nat. Acad. Sciences, vol. 4.

DUKE, E. 1915 Infant mortality. Results of a field study in Johnstown, Pa., based on births in one year. Children's Bureau, U. S. Dep't of Labor, Washington.

DUNCAN, B. S., AND DUKE, E. 1917 Infant mortality. Results of a field study in Manchester, N. H., based on births in one year. Children's Bureau, U. S. Dep't of Labor, Washington.

DÜSING, C. 1884 Die Regulierung des Geschlechtsverhältnisses bei Vermehrung der Menschen, Thieren und Pflanzen. Jen. Zeitschr. Naturwiss., Bd. 17.

DUTTON, A. S. 1910 The greater frequency of stillbirths and deaths under one year among males than females. Med. Press and Circular, vol. 89.

EASTMAN, P. R. 1919 The relation of parental nativity to the infant mortality of New York State. Amer. Jour. Diseases of Children, vol. 17.

GEDDES, P., AND THOMSON, J. A. 1901 The evolution of sex. Revised edition, London.

GOEHLERT, V. 1882 Ueber die Vererbung der Haarfarben bei den Pferden. Zeitschr. Ethnologie, Bd. 14.

GUYER, M. F. 1910 Accessory chromosomes in man. Biol. Bull., vol. 19.

HAMMOND, J. 1914 Some factors controlling fertility in domestic animals. Jour. Agr. Sciences, vol. 6.

HEAPE, W. 1909 The proportion of the sexes produced by white and coloured peoples in Cuba. Phil. Trans. Royal Soc., London, vol. 200.

HIRSCH, M. 1913 Ueber das Verhältnis der Geschlechter. Centralbl. für Gynäkologie, Bd. 37.

HOFFMANN, L. 1885 Mitteilung ueber Äbortus. Deutsche Zeitschr. für Tiermedizin, Bd. 11.

HUBER, G. 1915 The development of the albino rat, Mus norvegicus albinus. II. Abnormal ova: end of the first to the end of the ninth day. Jour. Morph., vol. 26.

HUNTER, E. B. 1918 Infant mortality. Results of a field study in Waterbury, Conn., based on births in one year. Children's Bureau, U. S. Dep't of Labor, Washington.

JACKSON, C. M. 1913 Postnatal growth and variability of the body and of the various organs in the albino rat. Am. Jour. Anat., vol. 15.

JENDRASSIK, E. 1911 Ueber die Frage des Knabengeburten-Ueberschusses und ueber andere Hereditätsprobleme. Deutsche mediz. Wochenschr., Bd. 37.

KING, HELEN DEAN 1913 Some anomalies in the gestation of the albino rat (Mus norvegicus albinus). Biol. Bull., vol. 24.

1915 On the weight of the albino rat at birth and the factors that influence it. Anat. Rec., vol. 9.

1916 On the postnatal growth of the body and of the central nervous system in albino rats that are undersized at birth. Anat. Rec., vol. 11.

1916 a The relation of age to fertility in the rat. Anat. Rec., vol. 11.

1918 Studies on inbreeding. I. The effects of inbreeding on the growth and variability in the body weight of the albino rat. Jour. Exp. Zool., vol. 26.

1918 a Studies on inbreeding. III. The effects of inbreeding, with selection, on the sex ration of the albio rat. Jour. Exp. Zool., vol. 27.

KING, HELEN DEAN, AND STOTSENBURG, J. M. 1915 On the normal sex ratio and the size of the litter in the albino rat. Anat. Rec., vol. 9.

KIRKHAM, W. B. 1916 The prolonged gestation period in suckling mice. Anat. Rec., vol. 11.

KROON, J. P. 1917 Iets over de verhouding der sterfte van mannen en vrouwen. Nederlandsch. Tijdschr. voor Geneskunde, B. 61.

LEWIS, C. J., AND LEWIS, J. N. 1906 Natality and fecundity. New York.

LILLIE, F. R. 1917 The free-martin; a study of the action of sex hormones in the foetal life of cattle. Jour. Exp. Zool., vol. 23.

MALL, F. P. 1908 A study of the causes underlying the origin of human monsters. Jour. Morph., vol. 19.

MORGAN, T. H. 1919 The physical basis of heredity. New York.

NICHOLS, J. B. 1907 The numerical proportions of the sexes at birth. Mem. Amer. Anthropological Assoc., vol. 1.

PINARD, A., ET MAGNAN, A. 1913 Sur la fragilité du sexe mâle. Comptes Rendus, T. 156.

PIKE, F. H. 1907 A critical and statistical study of the determination of sex particularly in human offspring. Amer. Naturalist, vol. 41.

PRINZING, F. 1906 Handbuch der medizinischen Statistik. Jena.

RAUBER, A. 1900 Der Ueberschuss an Knabengeburten und seine biologische Bedeutung. Leipzig.

SCHULTZ, A. H. 1918 Studies in the sex-ratio in man. Biol. Bull., vol. 34.

SLONAKER, J. R. 1912 The effects of a strictly vegetable diet on the spontaneous activity, the rate of growth, and the longevity of the albino rat. Pub. Leland Stanford Jr. Univ.

SOZINSKEY, T. 1885 Statistics and other features of still-births. Med. and Surgical Reporter, vol. 52.

STAHL, H., UND HENNEBURG, B. 1902 Ueber Rückbildungserscheinungen am graviden Säugetieruterus. Anat. Anz., Bd. 20.

TERRY, S. H. 1917 The secret of sex. New York.

WALDVOGEL, A. M. 1913 Statistische Bemerkungen zu den Geburten in der Kgl. Universitätsklinik in München in dem Zeitraum von 1892–1912. Inaug. Dissertation, München.

WHIPPLE, G. C. 1919 Vital statistics. New York.

WILCKENS, M. 1886 Untersuchung ueber das Geschlechtsverhältniss und die Ursachen der Geschlechtsbildung bei Haustieren. Biol. Centralbl., Bd. 6.

VON WINIWARTER, H. 1912 Études sur la spermatogenèse humaine. II. Hétérochromosome et mitoses de l'épithélium séminal. Arch. de Biol., T. 27.

Resumen por el autor, David M. Siperstein.
Universidad de Minnesota.

Los efectos de la inanición aguda y crónica sobre el desarrollo y la estructura del testículo de la rata albina.

El desarrollo postnatal normal del testículo ha sido estudiado por el autor en una serie de ratas albinas. Estos testículos fueron comparados con los de ratas sometidas a una alimentación deficiente durante varios periodos. Unas cuántas ratas fueron alimentadas con una dieta abundante después de una inanición prolongada. Los resultados más importantes de estos estudios son los siguíentes: I. En ratas de dos dias el testículo aumenta en peso a pesar de haber sometido al animal al hambre durante 48 a 50 horas, pero las mitosis disminuyen en número y el proceso normal de la diferenciación histológica cesa. 2. Durante el periodo de nutrición deficiente durante tiempo variable en ratas de tres semanas, la mitosis continúa en las células de los tubos seminíferos, pero el proceso de la espermatogénesis cesa en el estado de espermatocito primario. Los espermatocitos degeneran y son reabsorbidos. Las espermatogonias y células de Sertoli solamente presentan cambios degenerativos en los casos de inanición extrema. 3. La inanición aguda en las ratas adultas con pérdida del 30 a 47 por ciento del peso total produce cambios degenerativos en unos cuántos túbulos esparcidos irregularmente. Todos los demás túbulos presentan una estructura normal en apariencia. 4. La mitosis es muy persistente en el epitelio seminífero y tiene lugar aún en aquellos túbulos en los cuales casi todas las células aparecen más o menos degeneradas. 5. La alimentación abundante después de una inanición prolongada (comenzada a las tres semanas de edad y extendida sobre 12 a 20 semanas) da como resultado la vuelta rápida a la estructura histológica normal. 6. Durante el periodo regenerativo el autor ha comprobado la existencia de una hipertrofia definida del tejido intersticial e hiperplasia de las células intersticiales.

Translation by José F. Nonidez
Cornell Medical College. New York

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, FEBRUARY 7

THE EFFECTS OF ACUTE AND CHRONIC INANITION UPON THE DEVELOPMENT AND STRUCTURE OF THE TESTIS IN THE ALBINO RAT

DAVID M. SIPERSTEIN

Institute of Anatomy, University of Minnesota, Minneapolis

FOURTEEN FIGURES

CONTENTS

Material and methods 356
Observations 357
1. Normal postnatal histogenesis of the testis 357
2. Changes in young rats subjected to acute inanition 362
3. Changes in rats held at maintenance by underfeeding from three to ten weeks of age 364
4. Changes in young rats retarded by underfeeding for long periods beginning at three weeks of age 367
5. Changes in adult rats after acute inanition 369
6. Changes in rats refed after underfeeding beginning at three weeks of age and extending for various periods 372
Discussion 375
1. Origin and significance of polynucleated giant cells 375
2. Mitosis during inanition 375
3. Interstitial tissue during inanition 376
Summary 377
Bibliography 379

The immediate and the remote effects of inanition upon the young organism are subjects of interest and importance, especially at the present time. Experimental studies in this field have yielded much of importance to biology in general and to medicine in particular. It is very desirable to know more about the changes produced in the sex glands of malnourished or undernourished infants and children, and the resulting effects upon the reproductive system in later adult life. Inanition in adults may also occur either alone or associated with acute or especially with chronic wasting diseases, causing degenerative processes

in the sex glands. Recent work on vitamine deficiencies (by McCarrison, Allen, Dutcher, and others) indicates that the changes produced in the sex glands by this partial inanition resemble those found after general inanition. A study of the changes occurring in the histological structure of the testis of the albino rat during experimental inanition at various ages was therefore undertaken, and the results are embodied in the present paper.

MATERIAL AND METHODS

A large part of the material for this investigation was presented to me by Dr. C. M. Jackson, to whom the writer is also very much indebted for constant advice, aid, and criticism throughout the course of this study. The material comprises the testes of several albino rats (Mus norvegicus albinus) that had been subjected to inanition experiments in the Institute of Anatomy. The adult animals during acute inanition were allowed water only; but one of the adults was placed on total inanition, as were also the young rats (two days old) which were removed from the mother and given neither food nor water. Those on chronic inanition were fed restricted amounts of Graham bread soaked in whole milk for various periods. Some were refed fully after underfeeding for various periods. The animals were killed by chloroform and autopsied, the testes being weighed (without epididymis) and portions fixed for histological study. For convenience the material at my disposal is grouped as shown in table 1. In the first column, representing the individual rat numbers, the letters indicate the series (those autopsied by myself are 'Si'), the number following indicates the litter, while the number after the decimal point identifies the individual rat.

The testes had been fixed in either Zenker's, Bouin's, or Flemming's fixatives. Paraffin sections were cut at 2 to 10μ in thickness. The sections were stained chiefly with haematoxylin and eosin, Weigert's iron haematoxylin counterstained with Van Gieson's stain, or Heidenhain's iron-alum haematoxylin counterstained in some instances with orange G or acid

fuchsin. In general, Heidenhain's iron-alum haematoxylin without any counterstain gave by far the best results.

My own material was fixed in Carnoy's mixture (no. 1) and in Bouin's fluid. Fixation for one hour with Carnoy's mixture was found to be best. Sections were stained with Heidenhain's iron-alum haematoxylin with or without counterstain (acid fuchsin). This gave clear-cut karyokinetic figures in all stages of mitosis.

A number of measurements of cross-sections of tubules were also taken from the stained sections by means of a micrometer eyepiece. Sections of each testis containing the largest number of tubules cut in cross-section were chosen. In each section all the tubules showing a true cross-section (approximately circular) were measured. The results embodied in table 1 are therefore the average of measurements of a variable number of tubules, ranging from 8 to 44 in the individual cases. While the observations are relatively few in number, they are sufficient to indicate clearly any marked change in size.

In addition, a few (ten to fifteen) nuclei of the interstitial cells in both the control and the test rats were measured at various stages and the averages calculated. Only a sufficient number of testes was taken from each group to determine whether any marked change in the size of the nuclei had taken place as a result of the test conditions. The results are listed in table 1. No measurements of the cell body were attempted, on account of the irregularity of form. Measurements on the seminiferous epithelial cells were likewise found to be impracticable.

OBSERVATIONS

1. Normal postnatal histogenesis (group I)

In order to understand more fully the changes which inanition produces in the testis, it is essential to review briefly its normal histological structure and histogenesis as a basis for comparison.

Newborn (fig. 1). In microscopic sections of the testis in the newborn rat, the seminiferous tubules appear closely packed together at the periphery of the testis, while in the center of the

TABLE 1

Individual number, age and condition, gross body weight and weight of the testes in the albino rats used for histological study

RAT NUMBER	AGE, ETC.	FINAL BODY WEIGHT	WEIGHT OF TESTES	PER CENT OF BODY WEIGHT	AVERAGE DIAMETER OF TUBULES	AVERAGE DIAMETER OF NUCLEI OF INTERSTITIAL CELLS
Group I—Normal controls						
		grams	*grams*	*per cent*	*micra*	*micra*
Si 1.4	Newborn	5.1	0.0029	0.027	37.2	7.1
Si 2.5	4 days	7.1	0.0061	0.085	42.1	7.1
Si 1.2	4 days	7.0	0.0045	0.064	41.3	
Si 1.3	7 days	9.1	0.0200	0.219	50.5	6.9
Si 1.5	14 days	14.7	0.0780	0.529	65.3	
Si 6.0	21 days	20.6	0.0920	0.446	99.7	4.7
K 8.1	30 days	26.0	0.1154	0.443	88.1	
Si 12.1	35 days	38.7	0.2895	0.748	210.3	
Si 7.1	43 days	36.2	0.3940	1.088	235.0	
St 5.2	56 days	77.0			222.4	
Si 10.1	56 days	48.2	0.5460	1.112	175.7	4.5
Si 11.1	63 days	58.3	1.0030	1.720	204.5	4.6
Si 8.2	70 days	140.0	2.6930	1.923	257.8	
S 14.0	450 days	314.0	2.7545	0.877	323.7	4.5
Group II—Acute inanition in rats starting two days after birth						
Si 1.1	Starved 48 hrs.	4.01 (loss 25%)	0.0043	0.107	34.9	
Si 2.3	Starved 40 hrs.	4.21 (loss 21%)	0.0051	0.121	39.5	
Si 2.6	Starved 50 hrs.	4.10 (loss 25%)	0.0045	0.109	40.7	7.0

Group III—Held at constant body weight by underfeeding from three weeks of age						
St 9.36	Maint. 21–51 days	30.5	0.0958	0.31	100.3	
St 12.71	Maint. 21–67 days	21.2	0.1056	0.50	95.9	4.8
S 7.31	Maint. 21–70 days	31.4	0.3310	1.05	141.8	3.6
S 7.32	Maint. 21–71 days	28.8	0.2100	0.73	102.8	
S 11.65	Maint. 21–73 days	22.5 (R)	0.1860	0.83		
		(L)	0.0496	0.22	75.6	3.7
Group IV—Retarded by underfeeding for long periods beginning at three weeks of age						
St 44.1	Underfed 21–223 days	63.9	1.097	1.72	89.7	4.1
St 44.7	Underfed 21–418 days	40.9	1.187	2.90	91.2	4.4
St 44.4	Underfed 21–428 days	47.0	1.2968	2.75	120.4	4.3
M 29.109	Underfed 70–314 days	68.7	1.099	1.58	196.2	4.4
Group V—Adult acute inanition						
S 25.0	Starved 9 days	198.3 (loss 35%)	1.479	0.75	234.2	4.9
S 26.0	Starved 8 days	171.5 (loss 39%)	1.905	1.11	216.3	4.3
S 27.0	Starved 8 days	219.0 (loss 30%)	2.430	1.11	267.3	
Si 9.1	Starved 11 days	114.0 (loss 47%)	1.468	1.28	168.5	4.2
Group VI—Refed after underfeeding from three weeks to age indicated						
St 12.53	85 days. Refed 3½ days	44.9	0.2010	0.45	98.7	4.2
St 10.26	96 days. Refed 14 days	86.3	0.2672	0.31	123.4	5.3
S 12.52	109 days. Refed 37 days	117.4	1.1848	1.01	223.7	6.5
S 33.118	346 days. Refed 206 days	219.0	2.5182	1.14	291.1	

section the tubules are less compactly arranged. The tubules average 37.2μ in diameter. The intertubular spaces contain many small capillary vessels and mesenchymal cells. The tubules themselves consist chiefly of a single layer of parietal epithelial cells, which apparently correspond to those designated as 'Sertoli cells' by v. Ebner ('02) in the testis of the colt. These cells fuse to form a syncytium. Lumina have not yet appeared in the tubules. The central portion of each tubule contains from one to three large clear cells, each containing a definite, well-marked nucleus. Each nucleus has from one to three small clumps of chromatin situated usually (but not always) close to the periphery of the nucleus. These masses probably represent chromatin nucleoli. These large central cells apparently correspond to those described by v. Ebner ('02, fig. 1147) as the primitive germ cells ('Ursamenzellen') from which the spermatogonia are probably derived.

Interstitial cells (of typical structure) have not yet developed. There is present, however, in the intertubular tissue a peculiar type of cell which appears to be the forerunner of the later interstitial cells (fig. 14, *A*). This cell contains a large round nucleus (average diameter, 7.1μ) extremely rich in chromatin, which in turn is surrounded by a narrow rim of clear cytoplasm. By the form of its nucleus and its deeply staining chromatin, it is easily distinguished from the ordinary young mesenchymal or connective-tissue cell.

Fourth day (fig. 2). In a normal rat at four days of age the development of the testis has advanced considerably. The whole section is filled with seminiferous tubules (average diameter, 41.7μ). Within the tubules themselves the parietal cells show no material increase in number, but the spermatogonia (Allen's cell type A) have appeared. They are probably derived from the central cells ('Ursamenzellen' of v. Ebner), which still occur (less numerously) and present mitoses, though less frequently than in the newborn. Before dividing, they often appear to migrate from the center of the tubule toward its periphery and crowd in among the parietal cells. Some tubules already contain a few cells which resemble Allen's type B; that is, a

primary spermatocyte cell in its earliest phase. The forerunners of the interstitial cells are beginning to group themselves together in the intertubular spaces.

One week (fig. 3). A section of the testis from an albino rat at seven days shows an increase in the number and size of the tubules (average diameter, 50.5μ). (The larger number of tubules appearing in a cross-section of the testis may, however, be due to an increase in the length of the tubules rather than an actual increase in their number.) This represents the earliest stage described by Allen ('18). The tubules now contain two or three layers of cells. Very few central cells still remain, but spermatogonia (Allen's type A) are present in large numbers. There are very few type B cells. In other words, an undifferentiated condition of the spermatic epithelium still prevails. The syncytium (Sertoli syncytium) which fills the tubules at this stage is exceptionally well shown. The number of mitotic figures has again increased, so there are now as many figures in the field as there were at birth. The interstitial cells are more numerous and the amount of interstitial tissue is increased.

Two weeks (fig. 4). There is a large increase in the apparent number of seminiferous tubules, and their average diameter has increased to 65.3μ. There are many cells of types A and B, and some tubules even show Allen's type C (spermatocytes in which the chromatin masses become more diffuse than in type B and assume a 'woolly appearance'). A few cells have advanced as far as the leptotene stage. It is now fairly easy to distinguish between Sertoli cells and spermatogonia. The Sertoli cell has a nucleus with only a little chromatin material, in this way differing from the nucleus of the spermatogonium, which has a large amount of deeply staining chromatin.

Three weeks (fig. 5). Lumina are just beginning to appear in the closely packed tubules, whose average diameter has now increased to 99.7μ. Spermatogonia, cells of types A, B, and C, are numerous. A few tubules already contain primary spermatocytes showing mitotic figures. In many places, degenerating primary spermatocytes and spermatogonia are also present. This appears to be a normal physiological process, occurring in

the testis at all ages. No secondary spermatocytes or spermatids are to be seen. The interstitial tissue is assuming its adult structure (fig. 14, *B*). The nuclei average only 4.7μ in diameter, which is much smaller than at birth (7.1μ).

One month (fig. 6). The testis at this stage shows only a slight advance in development. It is distinguished by the large number of primary spermatocytes present. Spermatids have not yet appeared. The interstitial tissue shows no change.

Six to ten weeks. Sections of testes from forty-three to seventy days of age show a gradual increase in the size of the tubules (table 1) and in the number of mature sperm elements. Mature spermatozoa appear for the first time at the age of forty-three days. Their number increases so rapidly that at eight weeks (fig. 7) the histological structure of the testis differs in no important respect from that in the older rats. The interstitial cells show no noticeable change in structure.

Judging from the statements of Hewer ('14) and Allen ('18), there appears to be considerable variation in the age at which the various steps in the process of spermatogenesis occur in the albino rat. Hewer reported that there is no differentiation of primary spermatocytes from spermatogonia until the twenty-fifth day after birth. Allen found that his rats reached the pachytene stage at two weeks; while my material first shows this stage at three weeks. Hewer found that lumina first appear in the tubules at seven weeks. Allen demonstrated them in all rats fourteen days old. In my own rats fourteen days old I found no evidence of definite lumina, but they begin to appear at twenty-one days. Hewer stated that spermatozoa are not present in the lumina of the tubules until nine weeks after birth. Allen found spermatozoa in rats at the age of thirty-seven days, which corresponds closely with my observation at forty-three days.

2. Changes in young rats subjected to acute inanition (Group II)

A comparison of the testes of the normal control (at four days) with those of the test animals of same age (but lesser body weight) in table 1 shows but little apparent retardation in the growth

in weight of the gonads of the latter (average weight in two controls, 0.0053 gram; in three test rats, 0.0046 gram). Although the test rats were kept on total inanition for forty-eight to fifty hours, the testes have evidently maintained nearly normal growth, although the body weight has decreased 21 to 25 per cent.

The remarkably persistent growth of the testes in the starved rats is especially evident upon comparison with the newborn of the same litter (Si 1.4). The starved rats have decreased in body weight until they, although four days old, are about 20 per cent below the normal newborn. The testes, however, show a large increase in weight (in spite of the total inanition). The change in weight from 0.0029 to an average of 0.0046 grams represents an increase of 59 per cent. This is in general agreement with the results of Stewart ('18), who found that in rats kept at maintenance (constant body weight) by underfeeding from birth to (average) sixteen days the testis apparently increased about 374 per cent in weight. The smaller increase in the testes of my starved rats is probably due to the greater severity and shorter duration of the inanition.

My measurements indicate that this increase of 59 per cent in the weight of the testes is due almost entirely to the increased number of tubules seen in a section. The size of the tubules has remained about the same. There has perhaps been some retardation of the normal growth in size of the tubules. The normal testis at four days shows an average tubular diameter of 41.7μ, while the average diameter of the tubules in group II is only 38.4μ. This small difference, however, is of doubtful significance.

All three of the starved young rats (Si 1.1, Si 2.3, Si 2.6) show practically the same histological structure in the testis. The tubules are close together and more numerous than in the newborn, although there are relatively few in the center of the section. Cell differentiation has not progressed much beyond that found in the newborn. Each tubule presents the single layer of parietal cells. These cells are all alike and contain large nuclei, with very little chromatin material, surrounded by a

narrow strip of clear cytoplasm. The central cells ('Ursamenzellen' of v. Ebner) like those already described in the normal testis are also still present. There are very few mitotic figures either among the parietal cells or the central cells (fig. 8). Sections (stained with iron-alum haematoxylin) of the testes of controls show many mitotic figures in each field (figs. 1 and 2). This decrease in the number of karyokinetic figures is probably due to the condition of inanition. In general, the histological picture shows that the testis has advanced to the stage when type A cells are beginning to appear. Thus the stage of differentiation is somewhat beyond that corresponding to the normal at similar body weight, but behind that corresponding to the age or actual testis weight. The increase in the (apparent) number of tubules evidently accounts for the increase in the size (weight) of the testis.

Swingle ('18) similarly found that total starvation inhibits the growth and metamorphosis of Rana pipiens larvae. Spermatogenesis ceases and sex differentiation is prevented.

3. Changes in rats held at maintenance by underfeeding from three to ten weeks of age (group III)

In the five rats of this group held nearly at constant body weight by underfeeding from three to about ten weeks of age, the testes, though below normal weight for that age, have been retarded in their growth far less than has the body weight. Thus on comparing the average data for group III (table 1) with the normal control at three weeks (Si 6.0) it appears that the body weight has increased from 20.6 grams to 26.9 grams, an increase of about 31 per cent; while the testes have increased from 0.0920 gram to an average of 0.1955 gram, an increase of about 113 per cent. This would indicate a relative growth for the testis even greater than that (+34 per cent) found by Jackson ('15 a) under similar conditions. Individual variations in the control or test rats probably account for the difference.

The size of the tubules in group III has apparently increased from an average diameter of 99.7μ (normal at three weeks of

age) to an average diameter of 110μ. This increase in diameter (though of doubtful significance) would indicate that the increase in the weight of the testes is due in part to the increase in the diameter of the tubules.

With the exception of one rat (S 11.65), which will be mentioned later, the testes in this group all present the same microscopic picture (fig. 9). The tubules are placed close together. Lumina are just beginning to appear, although most of the tubules still show no central lumen. No spermatozoa or spermatids are present. There is no evidence to indicate that they had ever been developed and had undergone necrosis. A few secondary spermatocytes can be seen, but they are abnormal in structure.

Almost every primary spermatocyte also shows some degenerative changes. The cytoplasm becomes coarsely granular and contains clumps of basophilic staining material. Other cells show a fatty (?) degeneration or vacuolization of the cytoplasm (a positive statement cannot be made, as material stained for fat was not available). The nuclei degenerating by a process of karyorrhexis may give rise to the basophilic granules present in the cytoplasm of so many spermatocytes. The pycnotic nuclei are very abundant and, with progressive necrobiosis, appear gradually to lose their staining capacity, terminating in karyolysis. The cytoplasm also progressively loses its staining capacity and gradually disappears.

The spermatogonia, however, appear nearly normal. The Sertoli cells are increased in number, but show no structural changes. The tubules are filled with a structure resembling the 'degeneration reticulum' described by Kuntz ('20) in the testis after division of the nerves. This appears to me to be due largely to the Sertoli syncytium which becomes visible in direct ratio to the number of other cells which are being destroyed. Allen ('19) pointed out that in normal tubules the Sertoli syncytium is hard to see on account of the closely packed germ cells. As a result of experimental conditions, the syncytium is made more prominent by the removal of the germ cells which normally obscure it.

There are present in all cases many multinucleated or giant-cells. These cells are situated in the center of the lumen, and each tubule usually presents one or two of them. Each cell may contain from two to four nuclei, all showing some degenerative changes. Multinucleated cells appear to be very characteristic of the degenerative process in the testis. To a certain extent, the degree of severity of the starvation can be measured by the size of these cells and the number of nuclei they contain. For example, absolute inanition for nine days (in the adult) produces giant-cells containing as high as twelve to fifteen nuclei, while in the slower chronic inanition in the present group, the number is reduced to three or four nuclei.

One other important fact, which is strikingly illustrated in these sections, is the remarkable persistence of mitotic figures in the spermatogonia and primary spermatocytes. In rats which have been kept at constant body weight by underfeeding for seven weeks, the number and structure of the mitotic figures is apparently normal as compared with control rats (from three to four weeks). This fact is in agreement with the observations by many investigators (mentioned later) who have reported the presence of mitotic figures after prolonged chronic inanition. This mitosis is doubtless correlated with the persistent growth of the testis in the underfed young rats under these conditions, the new cells formed being in excess of those destroyed by degeneration.

My study of this group of albino rats kept at maintenance by underfeeding from three to ten weeks leads to the following conclusions: First, the chronic inanition prevents the testis from reaching the stage of spermatogenesis normal for the corresponding age. Second, in spite of the persistent mitosis and increased size of the testis, the process of spermatogenesis remains at the stage where spermatocytes are produced, corresponding to the normal stage at the age of three or four weeks. The growth capacity of the testis under these conditions of subnormal nutrition is apparently sufficient only to produce primary spermatocytes from the spermatogonia. Third, the spermatocytes degenerate and are absorbed, though not so rapidly as they are formed.

The excess of production over destruction partly accounts for the continued growth in weight of the testis. Fourth, the interstitial tissue is apparently unchanged and resembles in quantity and structure the tissue present in a normal rat at the age of three or four weeks.

In one rat (S 11.65, fig. 10) in this group, the testis shows such remarkable changes that it seems advisable to describe it separately. This was the left testis (indicated by (L) in table 1), which was markedly atrophic, weighing only 0.0496 gram. All the tubules show degenerative processes. Many contain no cells whatever. The lumina are filled with the characteristic 'degeneration reticulum,' and some have large vacuoles in their centers. Pycnosis, karyorrhexis, and karyolysis are prevalent.

A few of the seminiferous tubules in this case show a type of degeneration which differs entirely from any other thus far described. All the nuclei in every cell in the tubule gradually and almost uniformly lose their basophilic staining capacity and become acidophilic, taking the red counterstains. At the same time the cytoplasm of all the cells fuses together and becomes homogeneous in appearance. So that the entire cytoplasm in the tubules finally becomes one single mass, resembling very closely the homogeneous mass of necrotic tissue seen in an infarct. This mass apparently undergoes a process of autolysis, and vacuoles later replace the cytoplasmic substance. This process continues until finally the structure disintegrates and the position of the tubule is indicated by only the membrana propria. In spite of this necrosis in the tubules, the intertubular tissue shows no marked degenerative changes. However, a very irregularly distributed moderate hyperplasia of the interstitial cells is apparent (fig. 14, *D*). While not the typical picture produced by the chronic inanition in group III, this condition evidently corresponds to the advanced degenerative process in an extreme case.

4. Changes in young rats retarded by underfeeding for long periods beginning at three weeks of age (group IV)

In the three rats underfed from three weeks to 223 to 428 days of age (table 1) the body has approximately doubled in

weight, whereas normally it should have increased to about twelve times the initial weight. The testes are likewise of course far below norm for the corresponding age, though apparently considerably above the norm for the younger rats of similar body weight (rats Si 10 and Si 11; also Donaldson's Wistar norm). Stewart ('18), however, concluded that in rats underfed beginning at three weeks of age so as to reach only about 50 grams in body weight at 412 days of age the testes average 42 per cent below normal (for corresponding body weight). It is evident that the changes in the weight of the testis are extremely variable, so it is unsafe to draw general conclusions from only a few cases.

Judging from the size of the tubules in normal testes of similar weight, the tubules in group IV should average above 200μ in diameter. As shown in table 1, however, in three of the four cases the tubules appear subnormal (90 to 120μ) in diameter.

Microscopic sections (stained with haematoxylin and eosin) of the testis show that the size of the tubules in these rats long underfed has considerably decreased. The number of tubules has also decreased and they have become widely separated (fig. 11), due in part to the accumulation of fluid in the interstitial spaces (edematous infiltration). No mature spermatozoa or spermatids appear, although they are present in normal rats of corresponding body or testis weight. Spermatogenesis here (as in group III) has apparently not passed the primary spermatocyte stage. Most of the tubules contain three to four layers of cells, composed of spermatocytes and spermatogonia. The former show the most profound changes. Some appear as shadows without any nucleus or cytoplasm. Others have pycnotic nuclei and a homogeneous cytoplasm. Multinucleated giant-cells are present and the nuclei in these cells show pycnotic changes. Many spermatogonia and all the Sertoli cells appear normal. Mitotic figures are still present. Apparently some vascular changes have taken place. There is a slight sclerosis in the walls of the blood-vessels and also (apparently) a reduced blood-supply to the tubules. There is not any noticeable increase in the quantity of interstitial tissue (proper) (fig. 14, *E*) or any appreciable change in the structure of the interstitial cells, but the quantity of interstitial fluid has enormously increased.

Since all three rats (St 44.1, St 44.4 and St 44.7) show the same histological structure, the question of individual variation may be excluded. So that it appears that the 'growth tendency' so strongly manifested in the testis during inanition in young animals is confined to the formation of the earlier cells in the spermatogenetic cycle; that is, the process can go only to the spermatocyte stage. This is probably what happened in the case of the rats in group III, as before mentioned.

In one rat (M 29.109) inanition was started at ten weeks of age after the testis had presumably reached the stage when mature spermatozoa are produced. Even though kept at maintenance (constant body weight) by extreme underfeeding for 244 days, this testis shows the following histological structure. Most of the tubules are normal in structure and size and show well-developed spermatozoa. However, many lumina are filled with a débris made up almost entirely of heads and tails of spermatozoa. A few tubules show a slight desquamation process, but this is not distributed evenly among the tubules and is probably of no importance. Aside from this, the structure of the testis is apparently normal. Mitotic figures are present and all the various stages of spermatogenesis can be followed out. The conditions in this case emphasize the importance of the age factor. It indicates that if the testis has reached sexual maturity before experimental inanition, the effect upon spermatogenesis is much less marked than in cases where the underfeeding is begun at an earlier period, although the further growth of the testis (in weight) may be hindered. No final conclusions can be drawn from this one rat, however, although the results are in agreement with the conclusions of Jackson and Stewart ('20) as to the importance of the age at which the inanition begins.

5. Changes in adult rats after acute inanition (group V)

As shown in table 1 (group V), adult rats were subjected to acute inanition for periods varying from nine to twelve days. The loss in body weight varies from 30 to 47 per cent. The testes also apparently lose a great deal in weight, but the exact amount can of course be only roughly estimated. The test rats

at the beginning of the experiment varied in body weight from 216 to 328 grams. According to the Wistar norm tables compiled by Donaldson ('15), these body weights call for from 2.5 to 2.8 grams of testis. A comparison with the figures in table 1 shows that at autopsy the testes in weight varied from 1.468 to 2.43 grams, indicating considerable loss in weight. The average diameter of the tubules has correspondingly decreased from over 300μ to an average of 221.5μ. Jackson ('15) found that the loss in weight of the testis of adult rats during acute inanition is nearly proportional to that of the whole body. This agrees in general with the results obtained by Falck ('54), Voit ('66), Manassein ('68), and Gerhartz ('09). An atrophy of the testis relatively much greater than the decrease in body weight during inanition was noted in birds by Grandis ('89), and (during vitamine deficiency) by McCarrison ('19) and Dutcher ('20). A similar result in rats on lipoid-free diet was noted by Hatai ('15).

In one rat (Si 9) which was held for eleven days without food or water, the testis had shrunken away from the tunica albuginea and had decreased considerably in size. This was, however, not apparent upon removing the organ at autopsy, because the intervening space was filled with a fluid, which kept the tunic distended and gave it a normal rounded appearance. This phenomenon has likewise been noted by Allen ('19) in his study of rats subjected to a diet deficient in the water-soluble vitamines.

At first glance, the sections of the testes in this group appear perfectly normal in structure. The majority of the tubules are apparently normal in shape and show no signs of degeneration, as was likewise noted by Simonowitsch ('96). Spermatogenesis appears to be going on as usual. Spermatozoa are abundant and show no alteration in structure. There is no apparent change in the amount or structure of the interstitial tissue.

However, more careful study shows that in each section a few tubules have suffered as a result of the inanition. These tubules, though few in number and placed in immediate relationship with perfectly normal tubules, may show very marked and very severe changes (fig. 12). The spermatozoa are irregular in structure and show extreme degenerative processes. The sper-

matids may present a picture which strongly suggests a true synizesis. This peculiar clumping of the chromatin toward the periphery and toward one side of the nucleus has been described by Monterosso ('12) as a form of degeneration. But I have observed this same structure in almost everyone of my control rats, and believe that we are dealing here rather with a rearrangement of the chromatin material in the normal cytomorphosis of the cell.

The secondary and primary spermatocytes show marked necrobiotic changes. Even the spermatogonia are involved and reveal pycnotic and karyolytic changes in the nuclei. The Sertoli cells are apparently the only ones not severely injured.

Depending on the stage of degeneration, large numbers of multinucleated giant-cells are present in the tubules. These cells each contain from four or five nuclei up to as many as twelve or fifteen nuclei. Some tubules are so atrophic and degenerated that only the membrana propria remains, with a few necrotic giant-cells in the lumina. These giant-cells were carefully studied with special reference to their origin. Apparently they are derived from primary spermatocytes, which fuse together in groups during their desquamation. These cells will be referred to again at a later period.

Desquamation of the epithelial cells in these degenerating tubules is very common and apparently initiates the process of degeneration. This process appears to start with the secondary spermatocytes, but as the sloughing process continues the primary spermatocytes and even the spermatogonia are detached into the degenerating mass in the lumen of the tubule. Degeneration takes place in every case after desquamation has occurred. This degeneration apparently is also always in inverse order to that of spermatogenesis. The spermatozoa are the first to disappear, then the spermatids, followed by the spermatocytes and spermatogonia. The Sertoli cells are the most resistant. This agrees with the results obtained by Monterosso (in starved rats), Bouin and Garnier (in alcoholized rats), and Regaud and Tournade (after ligation of the spermatic cord). Allen ('19) states that (in degeneration due to vitamine deficiency) "the

spermatocytes seem to be the first affected, the spermatids next, and the spermatogonia last."

It therefore appears that acute inanition notably affects but comparatively few tubules in the testis, and that the other tubules retain their normal structure, but all the tubules suffer a loss in size. In spite of a loss of about one-third in the weight of the testis, spermatogenesis apparently continues normally in most of the tubules. But the few tubules which for some unexplainable reason are less resistant show atrophic and necrotic changes which are as marked as any found in chronic inanition.

Likewise, atrophic and degenerative changes in the seminiferous epithelium during inanition have been described by Grandis ('89) in pigeons, Pernice and Scagliosi ('95) in chickens, Simonowitsch ('96) in rabbits and guinea-pigs, Loisel ('01) in the dog, Monterosso ('12) in rat and mouse, and Poiarkoff ('13) in the dog. Similar changes have been described as a result of vitamine deficiency by McCarrison ('19) in pigeons and Allen ('19) in rats.

The extent of the degenerative changes naturally varies in general with the severity of the inanition. In the adult rabbit, Traina ('04) found that spermatogenesis ceases when the loss in body weight reaches about 30 per cent.

There is also a variation according to species, season, etc. At certain periods the testis during inanition may even continue to develop at the expense of the remainder of the body in the salmon (Miescher, '97) and frog (Nussbaum, '06).

6. Changes in rats refed after underfeeding beginning at three weeks of age and extending for various periods (group VI)

In one rat (St 12.53) refed for three and one-half days (table 1), the refeeding period was too short to produce any definite results. There is apparently already some increase in the total body weight, but very little, if any, in the testis. Sections show no definite change from the appearance of the testes in group IV. The primary spermatocytes still show necrotic changes and there is no advance in the stage of spermatogenesis. The

average diameter of the tubules (98.7μ) is slightly below that in rat S 7.32, with similar testis weight.

In another rat (St 10.26), refed for fourteen days after maintenance by underfeeding from twenty-one to seventy-two days, many improvements have taken place. The body weight has increased from around 30 to 86.3 grams, but there is no great increase in the weight of the testes. The reason for this becomes more evident upon histological study. Sections (stained with iron haematoxylin) show no increase in the number and but slight increase in the diameter of the tubules, compared with those of group III. But all the débris has been removed and no degenerating cells are to be seen (fig. 13). No secondary spermatocytes are present. The interstitial cells and their nuclei appear normal (but increased in size). They are, however, definitely increased in number above normal. They are evenly distributed throughout the testis, in this way differing from the condition found by Allen ('19) in the rats of Osborne and Mendel subjected to a diet deficient in the water-soluble vitamines. There are numerous mitotic figures among the spermatocytes and spermatogonia, showing all the stages of the karyokinetic cycle. The blood-vessels are distended with blood. It would appear that all the energy in the testis has been utilized to 'clean up' the waste material and to prepare for a further advance. This may account to some extent for the lack of increase in the weight of the testis.

In the rat (S 12.52) which was refed for thirty-seven days, the weight of the testis has greatly increased and histological study shows that spermatogenesis is going on normally. The tubules are normal in structure and contain many mature spermatozoa. The average diameter of the tubules has, however, increased to 222.7μ, accounting for the increase in weight. The only difference to be found (as compared with normal control testes) is an increase in the amount of interstitial tissue (fig. 14, *F*).

We must distinguish between interstitial tissue and interstitial cells. The former term applies to all tissues outside of the tubules proper; the latter (interstitial cells) refers only to the

specific cells playing a part in the internal secretion of the testes. In reality, there is not so much a general hypertrophy of the interstitial tissue, but rather a hyperplasia of the interstitial cells proper. There is a marked increase in the number of these cells and a slight increase in their size. They assume a more or less cord-like arrangement. The nuclei of the cells are spherical and rather vesicular, although a few clumps of chromatin are always present close to the periphery. A light granular cytoplasm surrounds the nuclei. After the very young stages (one to seven days), mitosis among these cells has never been observed. One possible explanation which may be offered is that mitosis in these cells takes place very rapidly, so that, although cell division occurs, it is rarely found in the sections.

The fourth rat in this group (S 33.118), refed for 206 days, has completely recovered from the effects of the underfeeding and presents a perfectly normal adult histological structure. The diameter of the tubules shows an increase of 291.1μ. Even the interstitial tissue and cells appear normal in quantity and structure.

These results are in accord with the work of Stewart ('15), who found that in rats refed after underfeeding from three to twelve weeks of age, the testes remain somewhat below normal weight for a few weeks, but regain normal weight before the adult stage is reached. In later experiments (Stewart, '18), in which the underfeeding was prolonged, the testis became markedly subnormal in weight (as found by Jackson, '15 a). Jackson and Stewart ('19) reported that the testis, which increases in relative weight in young rats during underfeeding, remains somewhat above normal weight during refeeding to a body weight 25 to 50 grams, but is subnormal in those refed to 75 grams body weight. Finally, Jackson and Stewart ('20) found that when the underfed rats are fully refed to maximum body weight, the testes are definitely above normal for the corresponding body weight. A restoration of the testis to normal structure and function upon refeeding after inanition has likewise been noted by Simonowitsch ('96) in rabbits and guinea-pigs, and by Loisel ('01) and Poiarkoff ('13) in dogs.

DISCUSSION

1. Origin and significance of polynucleated giant cells

It has been claimed by some authors that giant-cells may occur normally in the testis. I have never seen either a polynucleated or a giant-cell in a normal seminiferous tubule. Monterosso ('12) found that these cells are very rare in normal tubules, but occur more frequently during early inanition. He found that their number increases with the length of inanition, and that as many as fifteen nuclei might be seen in a single cell. He believes that they are formed by a fusion of the neighboring cells.

On the other hand, Bouin and Garnier ('00) believe that these cells are formed by an abnormal mitosis of the nuclei within the cell. Allen ('19) noticed these cells during the early stages of degeneration, but offered no explanation as to their origin.

My studies lead me to believe that the multinucleated cell is formed by a fusion of primary spermatocyte cells when they begin to degenerate. As a rule, these cells show marked nuclear and cytoplasmic degenerative changes later than those cells which break off independently and do not fuse with other cells. This suggests that fusion of cells may be a sort of protective measure from the effects of the inanition. De Bruyne ('99), however, claims that these polynuclear cells are precursors of degeneration. No mitotic figures have ever been observed in any polynucleated giant-cell.

2. Mitosis during inanition

As to the occurrence of mitosis during inanition, my own observations agree in general with those of Morpurgo ('88, '89), Grandis ('89), Traina ('04), and others. Traina, for instance, found that mitotic figures persist after a loss in body weight of 30 to 35 per cent. On the other hand, Pernice and Scagliosi ('95), in young chickens on water inanition, and Morgulis, Howe, and Hawk ('15), in fasting dogs, reported an absence of mitosis in the testis. In my adult rats subjected to acute inanition

(S 25, S 26, S 27), with loss in body weight of 30, 35, and 39 per cent, mitotic figures are still present in large numbers. Another rat (Si 9), with loss in body weight of 47 per cent, still shows numerous mitotic figures. They are present even in tubules showing extreme degenerative changes. In young rats underfed from the age three to ten weeks, mitosis is apparently unaffected. Even in those animals in which the underfeeding was continued to 400 days or more, the mitotic figures can be seen in many of the tubules. It would therefore appear justifiable to conclude that it is impossible by inanition to suppress mitosis entirely in the epithelium of the seminiferous tubule. The mitoses, however, may be decreased in number, as found in the rats two days old subjected to acute inanition.

The presence or absence of amitosis in the testis has long been a disputed question. Monterosso ('12) found amitosis present among degenerating spermatocytes and spermatogonia. Allen ('18, '19) believes that amitosis does not occur. I have likewise never seen a definite case of amitosis in any of my sections.

3. Interstitial tissue during inanition

Some investigators mention the increased quantity of interstitial tissue, which is occasioned by any degeneration or atrophy of the seminiferous epithelium. Cordes ('98) found an increase in interstitial tissue in cases of chronic disease with cachexia. Kyrle ('10) and Voss ('13) pointed out that in diseased conditions and in underdeveloped testes, the tubules are far apart and the interstitial tissue surpasses the tubules in amount. Allen ('19) also found an increase in the quantity of interstitial tissue in rats fed upon a diet deficient in water-soluble vitamines.

In my own series of albino rats, there appears in no case any demonstrable increase in interstitial tissue during either acute or chronic inanition. However, in those rats which were refed after various periods of underfeeding (group VI), there is a gradual increase in the relative amount of interstitial tissue (interstitial cells) until the gland regains its normal size and structure. Thereafter, the interstitial tissue apparently de-

creases in amount until it reaches the normal proportion. That is, the interstitial cells appear relatively increased in number only during the regenerative period.

Measurements of the nuclei of interstitial cells indicate that there is a sharp decrease in the average diameter of the nucleus from 7.1μ at birth to 4.7μ at three weeks of age. From the three-weeks stage there is very little change in the size of the nucleus. Comparison with the test groups indicates that the size of the nucleus of the interstitial cells is not affected to any marked degree by the inanition tests. In group III, two of the three specimens measured show a decreased average diameter of the nucleus (in underfeeding from five to ten weeks of age); and in group VI, it would appear that refeeding causes some increase in the size of the nucleus, in addition to the numerical increase already noted. There is considerable individual variation, however, and the number of observations is insufficient to warrant final conclusions.

SUMMARY

The results of the present investigation may be summarized as follows:

1. In the normal testis of the albino rat during the first postnatal week the (solid) seminiferous tubules consist of a single layer of parietal cells ('Sertoli cells' of v. Ebner) and a few central cells ('Ursamenzellen' of v. Ebner). After the first week, the normal postnatal development of the testis and the process of spermatogenesis proceed essentially as described by Allen ('18).

2. In rats two days old starved for forty-eight to fifty hours, the testis increases markedly in weight, but mitoses are reduced in number and the normal process of histological differentiation is arrested. The seminiferous tubules remain nearly normal in diameter.

3. During underfeeding for various periods beginning in rats three weeks old, mitosis continues in the cells of the seminiferous tubules, but the process of spermatogenesis is arrested at the primary spermatocyte stage, which persists even in rats over

400 days old. The spermatocytes degenerate and are resorbed, but if the number formed exceeds those destroyed, the testis may increase in weight. Multinucleated giant-cells are formed during the process of degeneration. The spermatogonia and Sertoli cells usually persist unaffected, except in very extreme cases, where a complete degeneration and disintegration of the seminiferous epithelium may occur. If the underfeeding begins after sexual maturity, the seminiferous tissue is much more resistant and normal spermatogenesis may persist for a long time. The seminiferous tubules may increase slightly in diameter in the shorter tests, but usually appear subnormal in size in the longer experiments.

4. Acute inanition in adult rats, with 30 to 47 per cent loss in body weight, produces degenerative changes in a few, irregularly scattered, tubules. All the other tubules show apparently normal structure and spermatogenesis, although there is a general decrease in their size. The degenerative changes are initiated by a desquamation of the epithelial cells into the lumen of the seminiferous tubule, followed by pycnosis and karyolysis. The process involves first the spermatids and spermatozoa, then the secondary and primary spermatocytes, and finally the spermatogonia. The Sertoli cells are the most resistant. During the degenerative process, multinucleated giant-cells arise, apparently by fusion of the degenerating spermatocytes.

5. During inanition, mitosis is very persistent in the seminiferous epithelium, both in young and adult rats. It may occur even in tubules where nearly all the cells are more or less degenerated. Amitosis was not observed. A condition resembling synizesis was frequently observed in the spermatids in both controls and test rats.

6. Refeeding after prolonged inanition (beginning in rats at three weeks of age and extending to twelve to twenty weeks) results in a rapid improvement in the structure of the testis, although it may lag behind in weight for a while during the preliminary stages of reconstruction. Spermatogenesis returns to normal in a short time, the tubules gradually increase to normal diameter, and spermatozoa appeared in thirty-seven days.

7. There is a definite hypertrophy of the interstitial tissue and an increase in the number of interstitial cells of the testis during the regenerative period on refeeding after inanition (in growing rats). No hypertrophy of the interstitial tissue was found accompanying atrophy of the seminiferous epithelium during inanition in either young or adult rats. The structure of the interstitial tissue and the size of the nuclei apparently remain nearly normal during acute and chronic inanition, except in extreme cases, where degenerative changes in the cells may occur.

BIBLIOGRAPHY

Allen, Ezra 1918 Studies on cell division in the albino rat (Mus norvegicus albinus). III. Spermatogenesis: The origin of the first spermatocytes and the organization of the chromosomes, including the accessory. Jour. Morph., vol. 31, pp. 133–186.

1919 Degeneration in the albino rat testis due to a diet deficient in the water-soluble vitamine, with a comparison of similar degeneration in rats differently treated and a consideration of the Sertoli tissue. Anat. Rec., vol. 16, pp. 93–117.

Bouin et Garnier 1900 Altérations du tube séminifère au cours de l'alcoolisme expérimental chez le rat blanc. C. R. Soc. Biol. (Cited by Monterosso, '12.)

Cordes, H. 1898 Untersuchungen über den Einfluss acuter und chronischer Allgemeinerkrankungen auf die Testikel, speciell auf die Spermatogenese, sowie Beobachtungen über das Auftreten von Fett in den Hoden. Virchow's Arch., Bd. 151.

De Bruyne 1899 Signif. physiol. de l'amitose. C. R. Ass. Anat. 1re session. (Cited by Monterosso, '12.)

Donaldson, H. H. 1915 The rat. Reference tables and data for the albino rat and the Norway rat. Memoirs of The Wistar Institute of Anatomy and Biology, no. 6.

Dutcher, R. Adams 1920 The nature and function of the antineuritic vitamine. Proc. Nat. Acad. Sci., vol. 6, pp. 10–14.

v. Ebner, V. 1902 In Kölliker's Handbuch der Gewebelehre, 3. Bd., 2. Hälfte, S. 415–416.

Falck, C. P. 1854 Beiträge zur Kenntnis der Wachsthumsgeschichte des Thierkörpers. Virchow's Arch, Bd. 7, S. 37.

Gerhartz, H. 1909 Geschlectsorgane und Hunger. 2. Mitteil. Centralbl. f. Physiol., Bd. 22, S. 65–66.

Grandis, V. 1889 La spermatogenèse durant l'inanition. Arch. ital. de Biol., T. 12, pp. 680–682.

Hatai, S. 1915 Growth of the body and organs in albino rats fed with a lipoid-free ration. Anat. Rec., vol. 9, pp. 1–20.

JACKSON, C. M. 1915 Effects of acute and chronic inanition on the relative weights of the various organs and systems of adult albino rats. Am. Jour. Anat., vol. 18, pp. 75–116.

1915 a Changes in the relative weights of the various parts, systems and organs of young albino rats held at constant body weight by underfeeding for various periods. Jour. Exp. Zoöl., vol. 19, pp. 99–156.

JACKSON, C. M., AND STEWART, C. A. 1918 The effects of underfeeding and refeeding upon the growth of the various systems and organs of the body. Minnesota Medicine, vol. 1, pp. 403–414.

1919 On the recovery of normal weight in the various organs of albino rats upon refeeding after underfeeding from birth for various periods. Amer. Jour. Diseases of Children, vol. 17, pp. 329–352.

1920 The effects of inanition in the young upon the ultimate size of the body and of the various organs in the albino rat. Jour. Exp. Zoöl., vol. 30, pp. 97–128.

KUNTZ, ALBERT 1919 Degeneration of testis in the dog. Anat. Rec., vol. 17.

KYRLE, J. 1910 Ueber Entwicklungsstörungen der männlichen Keimdrüsen im Jugendalter. Wiener klin. Woch., Bd. 23.

LOISEL, GUSTAVE 1901 Influence du jeûne sur la spermatogénèse. C. R. Soc. d. Biol., T. 53, p. 836.

MANASSEIN, W. 1868 Zur Lehre von der Inanition. Centralbl. f. d. med. Wissensch. (Berlin), Bd. 6, pp. 273–275.

MCCARRISON, R. 1919 The pathogenesis of deficiency disease. Indian Jour. Med. Research, vol. 6, no. 3, pp. 275–355. (Also in Brit. Med. Jour., 1919, vol. 1, p. 177, and vol. 2, no. 3033.) (Abstracted in Jour. Am. Med. Ass., vol. 74, no. 12.)

MIESCHER, FR. 1897 Histochemische und physiologische Arbeiten, Leipzig, Bd. 2, S. 116–217.

MONTEROSSO, BRUNO 1912 L'azione del diguino e dell'estratto secco di tiroide sulla struttura dell'epitelio del tubo seminifero del topo. Archiv. de Biol., T. 27, pp. 35–62.

MORGULIS, S., HOWE, PAUL E., AND HAWK, P. B. 1915 Studies on tissues of fasting animals. Biol. Bull., vol. 28, pp. 397–406.

MORPURGO, B. 1888 Sul processo fisiologico di neuformazione cellulare durante la inanizione acuta dell'organismo. Arch. per le sc. med., T. 12, pp 395–418. (Also in Arch. ital. de Biol., 1888, T. 11, pp. 118–133.)

1889 Ueber den physiologischen Zellneubildungsprocess während der acuten Inanition des Organismus. Ziegler's Beiträge zur path. Anat. u. allg. Path., Bd. 4, S. 315–335.

NUSSBAUM, E. 1906 Fortgesetzte Untersuchungen über den Einfluss des Hungers auf die Entwicklung der männlichen Geschlechtsorgane der Rana fusca. Anat. Anz., Bd. 29, S. 315–316.

PERNICE UND SCAGLIOSI 1895 Ueber die Wirkung der Wasserentziehung auf Thiere. Virchow's Arch., Bd. 139, S. 155–184.

POÍARKOV, E. 1913 L'influence de jeûne sur le travail des glandes sexuelles du chien. (Communication préliminaire.) C. R. Soc. Biol. Paris, T. 74, pp. 141–143.

REGAUD ET TOURNADE 1903 Notes hist. sur les phénom. régressifs détermines dans le test. par l'oblitération du canal déférent. C. R. Soc. de Biol. (Cited by Monterosso, '12.)

SIMONOWITSCH, J. 1896 Ueber pathologisch-anatomische Veränderungen der Hoden bei vollständigem und unvollständigem Hungern der Thier und Auflütterung nach dem Hungern. (Cited by Mühlmann—Russiche Literatur über die Pathologie des Hungerns. Centralbl. f. allg. Path. u. Path. Anat., Bd. 10, S. 160.)

STEWART, C. A. 1916 Growth of the body and of the various organs of young albino rats after inanition for various periods. Biol. Bull., vol. 31, pp. 16–51.

1918 Changes in the relative weight of the various parts, systems, and organs of young albino rats underfed for various periods. Jour. Exp. Zoöl., vol. 25, pp. 301–353.

SWINGLE, W. W. 1918 The effect of inanition upon the development of the germ glands and germ cells of frog larvae. Jour. Exp. Zoöl., vol. 24, pp. 545–565.

TRAINA, R. 1904 Ueber das Verhalten des Fettes und der Zellgranula bei chronischem Marasmus und akuten Hungerszuständen. Ziegler's Beiträge zur path. Anat. u. allg. Path., Bd. 35, S. 1–93.

VOIT, CARL 1866 Ueber die Verschiedenheiten der Eiweisszersetzung beim Hungern. Zeitschr. f. Biol., Bd. 2, S. 308–365.

VOSS, H. 1913 Zur Frage der Entwicklungsstörungen des kindlichen Hodens. Centralbl. f. allg. Path., etc., Bd. 24, No. 10, S. 433.

FIGURES AND EXPLANATION

The drawings were made with the aid of a camera lucida. The unit of magnification is 630 diameters for all the figures, with the single exception of figure 10, which has a magnification of 127 diameters. All the material stained with Heidenhain's iron-alum haematoxylin had been fixed in Carnoy's fluid (no. 1). The remaining material had been fixed in either Bouin's or Zenker's fixatives.

PLATE 1

EXPLANATION OF FIGURES

Figures 1 to 7 show normal development in the epithelial wall and the growth of the tubules in albino rats from birth to eight weeks of age. All × 630.

Figures 8 to 13 show changes in the seminiferous epithelium and size of the tubules brought about by experimental conditions.

1 Transverse section of a tubule from the testis of a normal newborn rat (Si 1.4). Section 6μ thick; stained with iron-alum-haematoxylin and acid fuchsin. × 630. Note the presence of a single layer of parietal cells, *P.C.*, and the large central cells, *C.C.*, in the center of the tubule Each tubule is surrounded by a membrana propria, *M.P.*

2 Transverse sections of a tubule from the normal testis at four days (Si 2.5). Section 6μ thick; stained with iron-alum-haematoxylin. × 630. The number of parietal cells, *P.C.*, has increased, while the central cells, *C.C.*, are less frequent. Membrana propria, *M.P.*

3 Transverse section of a tubule from the normal testis at seven days (Si 1.3). Section 6μ thick; stained with iron-alum-haematoxylin. × 630. Mitotic activity is apparent. A spermatogonium in metaphase is well shown, *Sp.C.* Germinative cells are all alike (Allen's type A). Membrana propria, *M.P.*

4 Section of a tubule from the normal testis at fourteen days (Si 1.5). Section 6μ thick; stained with iron-alum-haematoxylin and acid fuchsin. × 630. Cells of (Allen's) types A and B are abundant and a few cells of type C are present, *A.*, *B.*, *C.* Sertoli cells, *S.C.*, are beginning to assume their adult position and structure. Membrana propria, *M.P.*

5 Section of a tubule from a normal testis of twenty-one days (Si 6.0). Section 10μ thick; stained with iron-alum-haematoxylin. × 630. All types of cells, *A.*, *B.*, and *C.*, are present. Primary spermatocytes, *P.S.*, have appeared. Sertoli cells, *S.C.* Membrana propria, *M.P.* Note the formation of a lumen, *L.*, in the tubule.

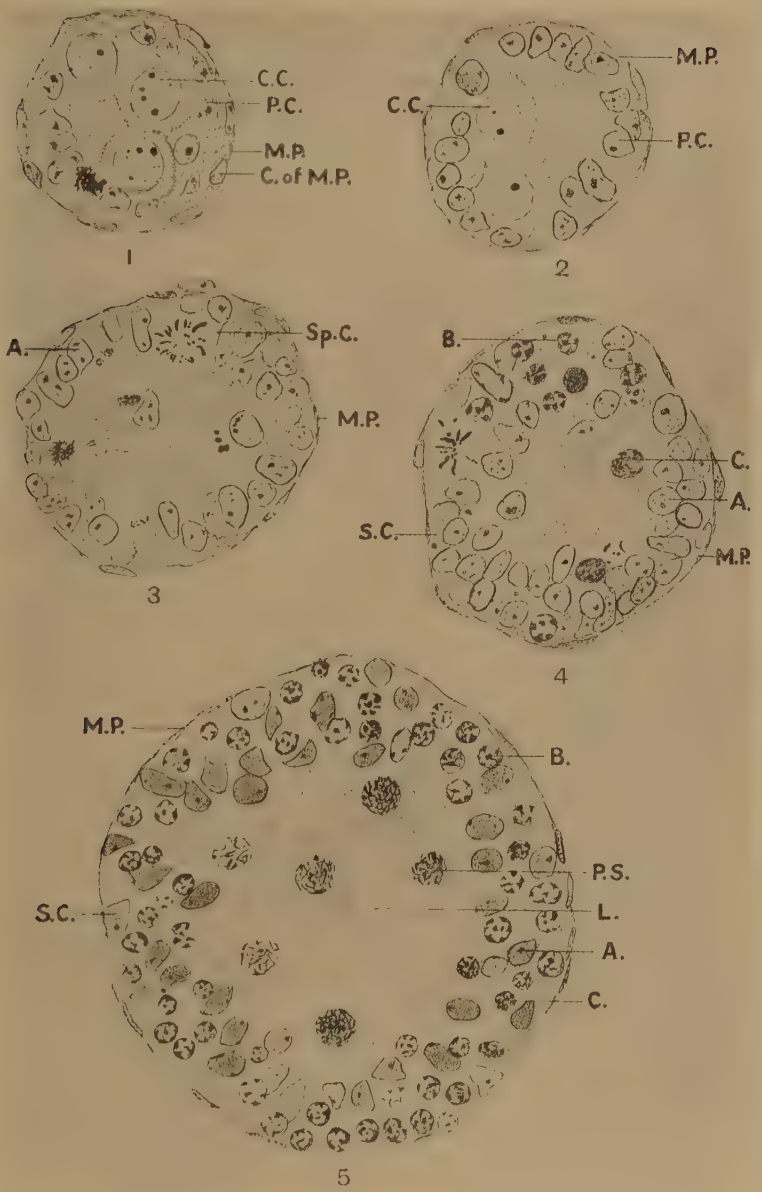

C.C.
P.C.
M.P.
C. of M.P.
1
M.P.
C.C.
P.C.
2
A.
Sp.C.
M.P.
3
B.
C.
A.
S.C.
M.P.
4
M.P.
B.
P.S.
S.C.
L.
A.
C.
5

PLATE 2

EXPLANATION OF FIGURES

6 Section of a tubule from a normal testis at four weeks (K 8.1). Section 10μ thick; stained with iron-alum-haematoxylin. × 630. Presents the same picture as the preceding figure, but also shows a marked increase in the number of primary spermatocytes, *P.S.*

7 Portion of a transverse section of a tubule from a normal testis at eight weeks (St 5.2). Section 10μ thick; stained with iron-haematoxylin. × 630. Note the gradual transition from the spermatogonia to the mature spermatozoa. This corresponds very closely to the condition found in the adult testis. Sertoli cell, *S.C.*; spermatogonia, *S.*; primary spermatocytes, *P.S.*; secondary spermatocytes, *S.S.*; spermatids, *Spt.*; spermatozoa, *Sp.*; membrana propria, *M.P.*

8 Section of three tubules from the testis of a rat four days old (Si 2.6) which had been subjected to acute inanition for fifty hours. Section 6μ thick; stained with iron-alum-haematoxylin and acid fuchsin. × 630. Note that the structure resembles that of the newborn more closely than that normal at four days. Compare with figures 1 and 2. Central cells, *C.C.*; parietal cells, *P.C.*; membrana propria, *M.P.*

B.
P. S.
S.C.
A.
M.P.
6
Sp.
Spt.
S.S.
P.S
S.C.
M.P.
S.
7
P.C.
C.C.
M.P.
8

PLATE 3

EXPLANATION OF FIGURES

9 Section of a tubule from a rat (S 7.32) held at constant body weight by underfeeding from three to ten weeks of age. Section 6μ thick; stained with haematoxylin and eosin. × 630. Degenerating primary spermatocytes, *D.P.S.*, showing the homogeneous, deeply acidophilic staining reaction of the cytoplasm. Note the persistence of mitotic figures, *M.F.*; the normal Sertoli cells, *S.C.*, and spermatogonia, *S.* A multinucleated cell, *Mu.C.*, typical of those appearing as a result of chronic inanition. Degenerating cytoplasm, *D.C.*; membrana propria, *M.P.*

10 Section of a portion of the atrophic testis from a rat (S 11.65) subjected to chronic inanition from three to ten weeks of age. Section 10μ thick; stained with haematoxylin and eosin. × 127. The central tubule shows the extreme stage of degeneration (described in text), a complete nearly homogeneous mass of necrotic tissue. The surrounding tubules show various stages of degeneration. Note the mitotic figures, *M.F.*, in the tubule to the right and the apparently normal Sertoli cells, *S.C.* A slight hyperplasia of the interstitial cells, *I.C.*, appears. Blood-vessel, *B.V.*

11 Portion of a tubule from the testis of a rat (St 44.4) stunted by underfeeding from three weeks to 428 days of age. Section 10μ thick; stained with haematoxylin and eosin. × 630. Note the degenerating primary spermatocytes, *D.P.S.*; the multinucleated cell, *Mu.C.*, in the lumen. Sertoli cells, *S.C.*, and spermatogonia, *S.*, are apparently normal. Membrana propria, *M.P.*

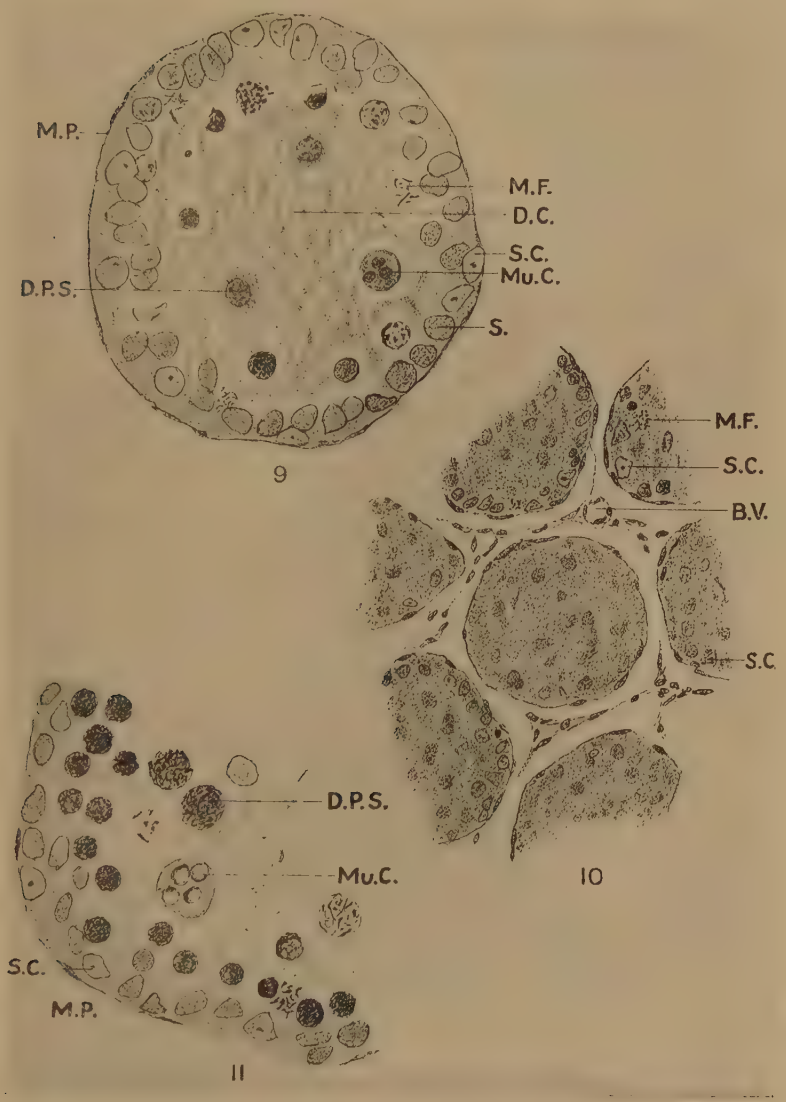
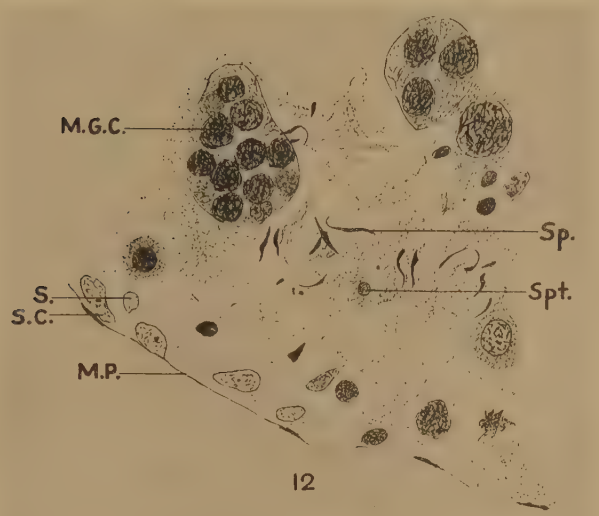

M.P.
M.F.
D.C.
S.C.
Mu.C.
D.P.S.
S.
9
M.F.
S.C.
B.V.
S.C
10
D.P.S.
Mu.C.
S.C.
M.P.
11

PLATE 4

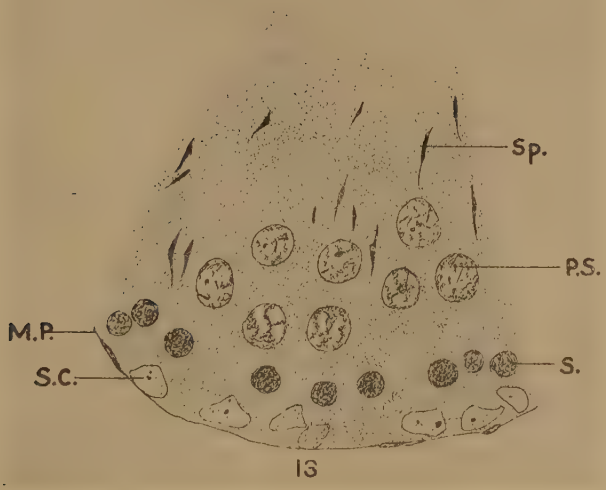
EXPLANATION OF FIGURES

12 Oblique section of a testis from an adult rat (S 25) subjected to acute inanition for nine days. Section 10μ thick; stained with haematoxylin and eosin. $\times$ 630. Multinucleated giant-cells, *M.G.C.*, occupy the center of the tubule. Débris composed of degenerating cells of various kinds fills the tubule. Spermatozoa, *Sp.*; spermatids, *Spt.*; spermatogonia, *S.*; membrana propria, *M.P.* The Sertoli cells, *S.C.*, still occupy their normal position, but show atrophic and degenerative changes.

13 Portion of transverse section of the testis from a rat (S 12.52) underfed from three to ten weeks of age and then refed for thirty-seven days. Section 7μ thick; stained with haematoxylin and eosin. $\times$ 630. Note the large number of normal primary spermatocytes, *P.S.*, and spermatozoa, *Sp.* Spermatogonia, *S.*, showing mitotic figures, are abundant. Sertoli cell, *S.C.*; membrana propria, *M.P.*



12

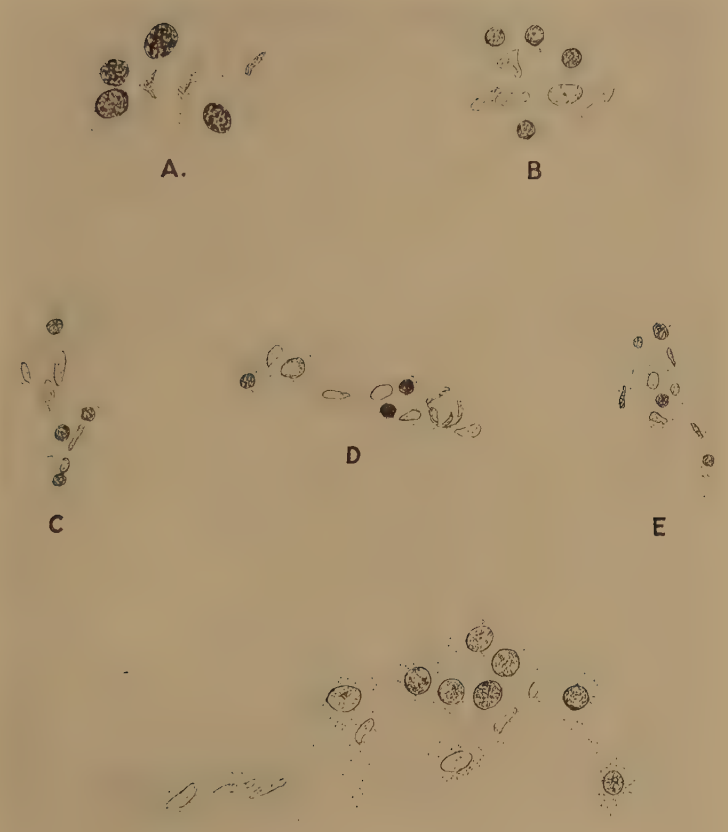


13

PLATE 5

EXPLANATION OF FIGURES

14 A to F represent various stages in the interstitial (mesenchymal) cells of the testis during normal development, inanition, and refeeding. All magnified × 630. *A*, normal newborn (Si 1.4). *B*, normal adult (S 14). *C*, underfeeding, three to ten weeks of age (S. 7.32). *D*, extreme atrophy in underfeeding from three to ten weeks of age (S 11.65). *E*, underfeeding from three weeks to 428 days of age (St. 44.4). *F*, refed thirty-seven days after underfeeding from three to ten weeks of age (S. 12.52).


A.
B
C
D
E
F
14

Resumen por los autores Bradley M. Patten y Rees Philpott.
Western Reserve University, School of Medicine.

La contracción de los embriones durante los procesos preparatorios a la obtención de cortes.

Para averiguar el grado de contracción producido al fijar material embriológico y prepararle para la obtención de cortes, los autores han llevado a cabo varias series de medidas en embriones de cerdo durante los diversos momentos de algunos de los procederes comunes. Los fijadores usados fueron los siguientes: Licores de Zenker, Orth, y Tellyesnicky; formol al 10%, formal-alcohol y licor de Bouin. Primero se midió la longitud del embrión desde la curvatura cefálica hasta la lumbar, cuando aquél estaba aún contenido en el líquido amniótico, y después a raíz del uso de cada una de las soluciones con que fué tratado. La contracción media fué medida en tantos por ciento de la longitud primitiva del embrión. Estas medidas fueron representadas gráficamente por curvas que indican el grado de contracción en cada uno de los momentos de la técnica empleada. La mayor contracción aparece después de la fijación con los líquidos que contienen bicromato, tales como los licores de Zenker, Orth y Tellyesnicky, y la disminución de la longitud del embrión varía desde el 30% en embriones de 10 mm. hasta próximamente el 20% en los embriones de 20 a 25 mm. El grado mayor de contracción en los embriones más jóvenes debe probablemente atribuirse a la menor compacidad de su mesodermo. La mayor parte de la contracción en estos métodos es causada por los fijadores. La fijación en formol al 10% y en formol alcohol produce un hinchamiento inicial, al cual sigue una contracción rápida durante la deshidratación. La contracción total después de la inclusión en parafina es próximamente igual á la producida por las soluciones de bicromato. La contracción después del licor de Bouin es ligera, pero continúa durante la deshidratación e inclusión. La contración total con este método es algo menor que la de los fijadores que contienen bicromato. La comparación de las diferentes gráficas indica una tendencia mayor hacia el aumento de la contracción durante la deshidratación despues de los fijadores que producen menos contracción inicial. Esto prácticamente anula, por los menos en el caso del material destinado a la inclusión en parafina, las supuestas ventajas de los fijadores mezclados con el fín de evitar la contracción por el fijador mismo.

Translation by José F. Nonidez
Cornell Medical College, New York

AUTHORS' ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, DECEMBER 27

THE SHRINKAGE OF EMBRYOS IN THE PROCESSES PREPARATORY TO SECTIONING

BRADLEY M. PATTEN AND REES PHILPOTT

Laboratory of Histology and Embryology of Western Reserve University, School of Medicine

EIGHT FIGURES

INTRODUCTION

The true or fertilization age of the human embryos which come into the laboratory is usually uncertain if not altogether unknown. For the most part the age of other mammalian embryos used for laboratory study is equally a matter of conjecture. For this reason it is customary to designate the stage of development attained by an embryo by giving its measurements. In this country the method of measuring embryos used by Mall is in quite general use (Keibel and Mall, '10, vol. 1, ch. 8).

If embryos always came into the hands of the investigator fresh, so that the measurements might be made under uniform condition, the methods in use would leave little to be desired, at least as far as establishing a basis for comparing different embryos is concerned. Unfortunately, however, embryos are brought into the laboratory in various stages of preservation or lack of it. The shrinkage of embryos under the various treatments to which they are subjected for preservation and in preparation for sectioning makes the comparison of embryos measured at different steps in the processes a precarious matter.

While the fact that shrinkage is to be expected is a matter of common information, we have been unable to find in the literature any quantitative data covering the shrinkage which is to be encountered in the various processes of fixation commonly in use for embryological material. The measurements

described in this paper were made to ascertain how much shrinkage occurs in the various steps of some of the more common methods of preserving embryos and preparing them for sectioning. Because linear dimensions are already in use as a criterion of the stage of development attained, they were used, rather than weight or volume, as a basis for determining the shrinkage. It is hoped that these measurements and the further accumulation of similar data will aid in more accurate comparisons of embryos which come into the hands of investigators in widely different stages and conditions of preservation.

MATERIAL AND METHODS

By reason of the readiness with which they could be secured fresh, and because of their close comparability with young human embryos, pig embryos were used for the entire series of measurements.

The embryos were measured first in amniotic fluid,[1] immediately on removal from the uterus. The measurements made were for the crown-rump length. The belly thickness was also measured in quite an extensive series as a check against the possibility that distortion of the spinal axis might be affecting the length measurements. Inasmuch as the shrinkage in per cent of the original dimensions corresponded fairly closely in the two cases, only the crown-rump measurements are here recorded. All the measurements were made with micrometer calipers graduated in millimeters and indicating the tenths of a millimeter by vernier.

In the early part of the work both of us measured the same embryos independently and compared our results. Our measurements tallied so consistently to the tenth of a millimeter that in the latter part of the series measurements were made by only one observer.

After the first measurement in the amniotic fluid, embryos were measured after each solution with which they were treated.

[1] Some embryos were measured also in physiological saline solution and their measurements compared with those made in the amniotic fluid. The measurements in the two fluids were identical.

Following paraffin infiltration the embryos were transferred from the molten paraffin to xylol for measurement, after which they were at once returned to paraffin for a few minutes before embedding. This process proved to be in no way detrimental to the infiltration or embedding.

Measurements were later carried out on several series of sagittal sections made from the embryos measured during the preliminary processes of preparation. When care is used in expanding the sections on the slide, their measurements correspond to the measurements made subsequent to paraffin infiltration.

MEASUREMENTS

Since the processes to which the embryos were subjected, and therefore the series of measurements made on them, differ in accordance with the fixing fluid, the data secured may be most simply presented under the heading of the fixing agent used.

Zenker's fluid (formula as given in Lee, '13)

The technique employed was not different from that commonly used with Zenker's fluid: twenty-four hours in fixative; overnight washing in running water; about four hours in each grade of the ascending alcohols up to 70 per cent; twenty-four hours in 70 per cent alcohol containing Lugol's solution for the removal of any remaining mercuric chloride; overnight in 95 per cent alcohol; four hours in absolute (two changes); three hours in xylol; two to three hours in soft, and two to three hours in hard paraffin, according to the size of the embryos.

The measurements made on Zenker-fixed embryos are given in detail in table 1 and summarized graphically in the curve of figure 1. To facilitate comparisons for variability, the material in table 1 has been arranged in the order of the lengths of the embryos when measured fresh in the amniotic fluid. It will be seen that while there is, as would naturally be expected, some individual variability, the range of the variation is small. Indeed, comparison of the measurements made on embryos of like original

TABLE 1

Measurements of pig embryos, showing the amount of shrinkage which occurs in the various solutions used in the Zenker method of preparing material for sectioning

IDENTIFICATION NUMBER OF EMBRYO	C.-R. LENGTH IN AMNIOTIC FLUID	ZENKER		WATER		35 PER CENT ALCOHOL		50 PER CENT ALCOHOL		70 PER CENT ALCOHOL AND LUGOL		95 PER CENT ALCOHOL		SECOND ABSOLUTE		XYLOL		SECOND PARAFFIN	
		C.-R. length	Shrinkage	C.-R. length	Shrinkage	C.-R. length	Shrinkage	C.-R. length	Shrinkage	C.-R. length	Shrinkage	C.-R. length	Shrinkage	C.-R. length	Shrinkage	C.-R. length	Shrinkage	C.-R. length	Skrinkage
	mm.	*mm.*	*per cent*	*mm.*	*per cent*	*mm.*	*per cent*	*mm.*	*per cent*	*mm.*	*per cent*	*mm.*	*per cent*	*mm.*	*per cent*	*mm.*	*per cent*	*mm.*	*per cent*
Z. a. 4	9.0	7.2	20.0	7.2	20.0	7.2	20.0	7.2	20.0	7.0	22.2	6.8	24.5	6.7	25.6	6.6	26.7	6.0	33.3
Z. a. 2	10.5	8.2	22.0	8.1	22.9	8.1	22.9	8.1	22.9	8.0	23.9	7.7	26.6	7.5	28.6	7.3	30.5	6.9	34.1
Z. a. 15	10.5	9.0	14.2	8.9	15.3	9.0	14.2	8.9	15.3	8.9	15.3	8.4	20.0	8.3	20.9	8.2	21.8	7.7	26.7
Z. a. 10	11.2	9.1	18.7	9.0	19.6	9.0	19.6	9.0	19.6	8.9	20.5	8.7	22.3	8.7	22.3	8.6	23.2	8.0	28.5
Z. a. 1	11.7	9.2	21.2	9.2	21.2	9.0	23.0	9.0	23.0	9.0	23.0	8.5	27.4	8.4	28.2	8.3	29.0	8.0	31.6
Z. a. 8	11.9	9.5	20.2	9.5	20.2	9.6	19.3	9.6	19.3	9.3	21.8	9.0	24.3	9.0	24.3	8.9	25.2	8.4	29.4
Z. a. 11	11.9	9.7	17.6	9.6	19.3	9.9	16.8	9.6	19.3	9.5	20 2	9.2	22.7	9.1	23.5	9.0	24.4	8.5	28.5
Z. a. 6	12.0	9.8	18.3	9.7	19.1	9.9	17.4	9.7	19.1	9.6	20.0	9.2	23.3	9.1	24.2	9.0	25.0	8.6	28.3
Z. a. 13	12.0	10.0	16.7	9.9	17.6	9.9	17.6	9.9	17.6	9.9	17.6	9.4	21.6	9.2	23.3	9.1	24.3	8.5	29.4
Z. a. 7	12.1	9.8	19.0	9.7	19.8	9.9	18.2	9.7	19.8	9.7	19.8	9.3	23.1	9.2	24.0	9.1	24.8	8.6	29.0
Z. a. 12	12.1	9.9	18.1	9.8	19.0	9.9	18.1	9.8	19.0	9.7	19.8	9.3	23.1	9.2	23.9	9.1	24.8	8.7	28.1
Z. a. 14	12.1	9.9	18.2	9.8	19.0	9.9	18.2	9.9	18.2	9.9	18.2	9.3	23.1	9.3	23.1	9.2	24.0	8.7	28.1
Z. a. 3	12.2	9.6	21.3	9.5	22.2	9.7	20.4	9.6	21.3	9.5	22.2	9.1	25.4	9.0	26.2	8.9	27.1	8.8	27.9
Z. a. 9	12.7	10.3	18.9	10.2	19.7	10.0	21.3	10.1	20.4	10.0	21.3	9.8	22.9	9.7	23.6	9.7	23.6	9.0	29.1
Z. a. 5	12.9	10.4	19.4	10.3	20.2	10.4	19.4	10.3	20.2	10.2	21.0	9.9	23.3	9.8	24.0	9.7	24.8	9.2	28.6
Z. c. 3	14.3	12.9	9.8	12.8	10.5	12.8	10.5	*	*	12.7	11.2	12.6	11.9	12.4	13.3	12.2	14.7	11.8	17.5
Z. c. 2	14.4	12.9	10.4	12.8	11.1	12.8	11.1	*	*	12.7	11.8	12.6	12.5	12.6	12.5	12.1	16.0	11.5	20.1
Z. c. 1	16.5	14.8	13.3	14.6	11.5	14.4	12.7	*	*	14.2	13.9	13.9	15.8	13.8	16.4	13.7	17.0	12.8	22.4

Z. c. 4	17.5	16.0	8.5	15.8	9.7	15.7	10.3	*	*	15.5	11.4	15.2	13.1	15.1	13.7	15.0	14.3	14.0	20.0
Z. c. 6	19.6	16.9	13.8	16.8	14.3	16.8	14.3	*	*	16.5	15.9	16.2	17.5	16.1	18.0	16.0	18.5	14.9	24.1
Z. b. 1	20.0	18.6	7.0	18.4	8.0	18.2	9.0	18.0	10.0	17.5	12.5	17.2	14.0	17.0	15.0	16.9	15.5	15.8	21.0
Z. b. 2	20.4	18.7	8.3	18.6	8.8	18.4	9.7	18.1	11.3	17.8	12.7	17.4	14.7	17.1	16.2	16.7	18.1	15.4	24.5
Z. c. 5	21.4	19.1	10.7	18.9	11.7	18.9	11.7	*	*	18.7	12.6	18.3	14.5	18.1	15.5	18.0	15.8	16.8	21.4
Z. c. 9	25.0	23.7	5.2	23.5	6.0	23.3	6.8	*	*	22.9	8.4	22.7	9.2	22.3	10.8	22.0	12.0	21.1	15.6
Z. c. 7	25.7	23.7	7.8	23.5	8.6	23.3	9.3	*	*	22.9	10.9	22.8	11.3	22.3	13.3	22.3	13.3	22.0	14.4
Z. b. 4	26.3	24.6	6.5	24.5	6.8	24.4	7.2	24.0	8.7	22.8	13.3	22.6	14.1	22.3	15.2	22.2	15.6	20.9	20.9
Z. b. 3	26.4	25.5	3.3	25.1	4.9	25.1	4.9	24.7	6.4	23.5	7.2	23.2	8.3	22.8	9.9	22.7	10.3	21.2	19.7
Z. c. 8	27.0	25.4	5.9	25.7	4.8	25.7	4.8	*	*	25.4	5.9	24.8	8.2	24.7	8.6	24.3	10.0	24.0	11.1
Average shrinkage in per cent.....		10.9		11.5		11.4		11.8		16.2		18.5		19.4		20.5		24.8	

* Not measured in 50 per cent alcohol.

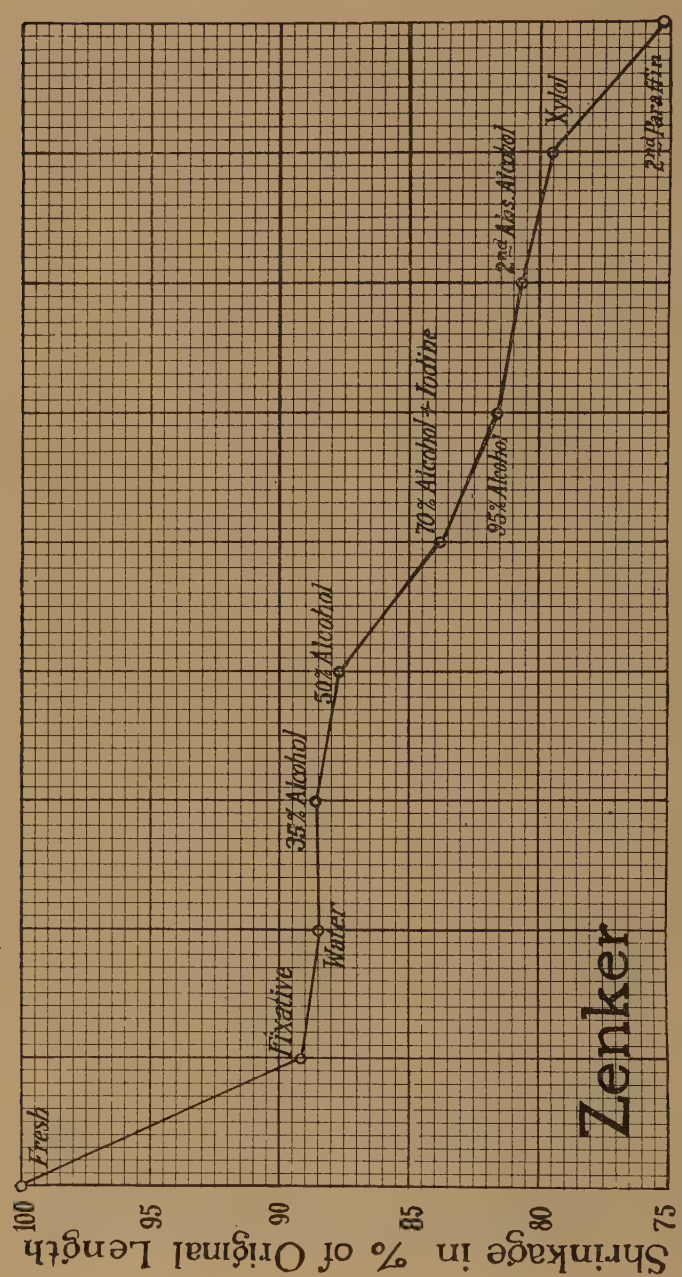


Fig. 1 Graph showing the shrinkage induced in pig embryos by the various solutions with which they are treated in the Zenker method of preparing material for sectioning. The extent of the shrinkage is indicated by expressing the embryos' length at various steps in the process as a per cent of their length when fresh. The points on the curve are located from the average measurements of twenty-eight embryos. The embryos when fresh ranged from 9 to 27 mm. in crown-rump length (average length fresh 16.1 mm.). Individual measurements for the same group of embryos are given in table 1.

length (e.g., embryos from 11.9 mm. to 12.2 mm., table 1) shows their shrinkage at various steps in the process to be surprisingly uniform.

The arrangement of the measurements in order of the original length of the embryos brings out another point. The total shrinkage is greater in the younger embryos than in the older. While it would be unsafe to draw any final conclusions from data based on embryos of the limited size range here studied, it seems probable that this difference in shrinkage is due largely to the differences in the organization of the developing muscular and connective tissues. In embryos of about 10 mm. the mesodermal tissues are represented for the most part by loosely aggregated cell masses with abundant interstitial spaces. By the time the embryo has attained a length of 20 to 25 mm. the cells of the developing muscular and connective tissues have become much more compact with a corresponding reduction of the interstitial spaces. Moreover, in the older embryos chondrification has begun in many centers and renders the body of the embryo more rigid as well as more compact. These conditions would seem quite sufficient to account for the fact that fixation and dehydration of very young embryos result in a greater shrinkage than that induced in older embryos.

The extent to which shrinkage varies with the original length of the embryo is shown graphically in figure 2. The curve is not as precisely indicated as it would be by a larger number of cases, but its general course is nevertheless quite apparent. Only measurements of embryos fixed in bichromate fluids were used in plotting this curve since their shrinkage is closely comparable, whereas some of the other fixing fluids show quite different shrinkage curves.

It was thought possible that preliminary coagulation of embryos by treatment in hot water might reduce the amount of shrinkage they suffered. To test this possibility, embryos were immersed for five minutes in water of 65°C. and then put through the routine Zenker process. As will be seen by comparing the curves of figures 1 and 3, this preliminary treatment with hot water proved to be ineffective in reducing the amount of shrinkage.

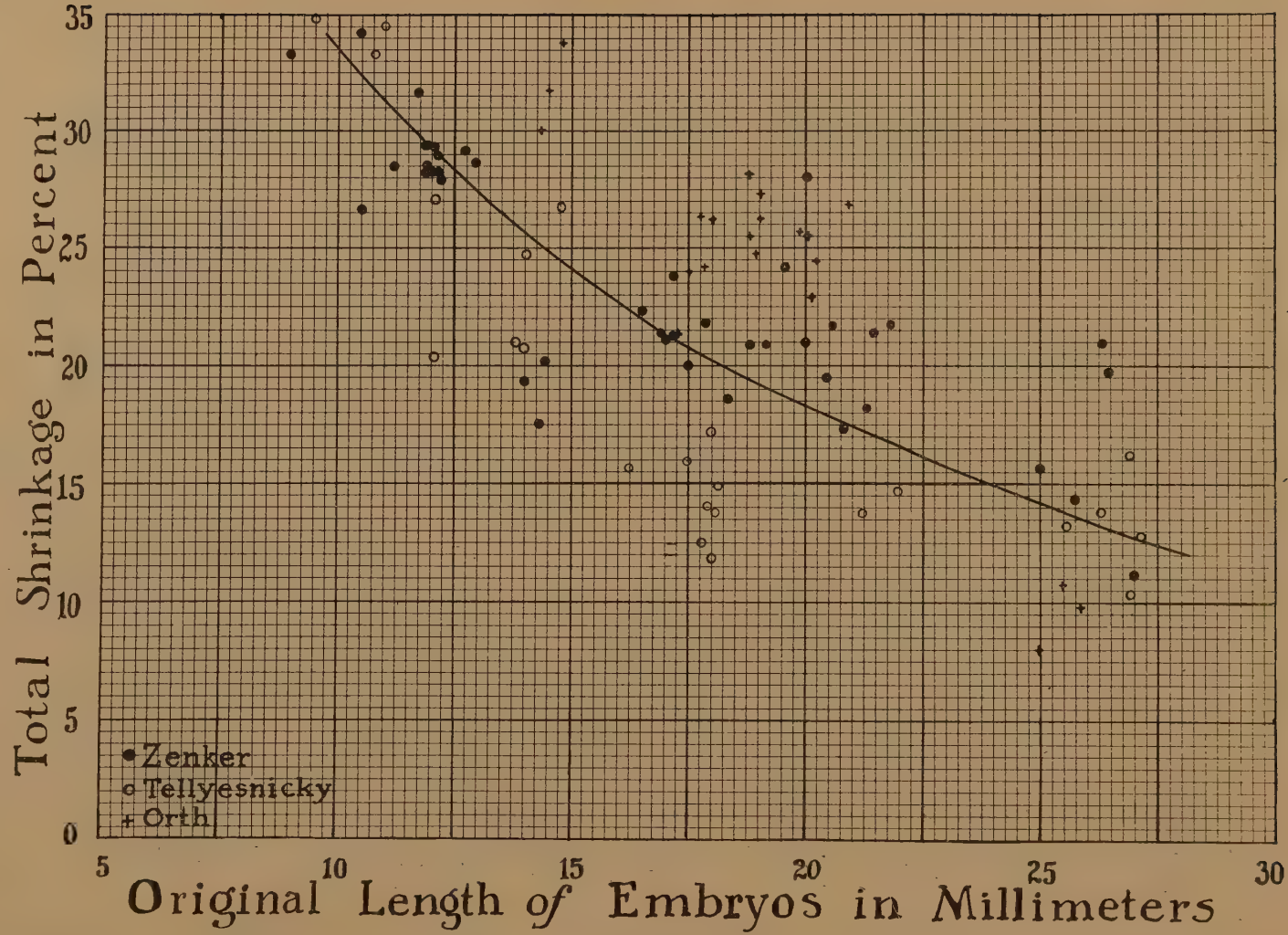

Total Shrinkage in Percent
35
30
25
20
15
10
5
0
5
10
15
20
25
30
Original Length of Embryos in Millimeters
Zenker
Tellyesnicky
Orth

Fig. 2

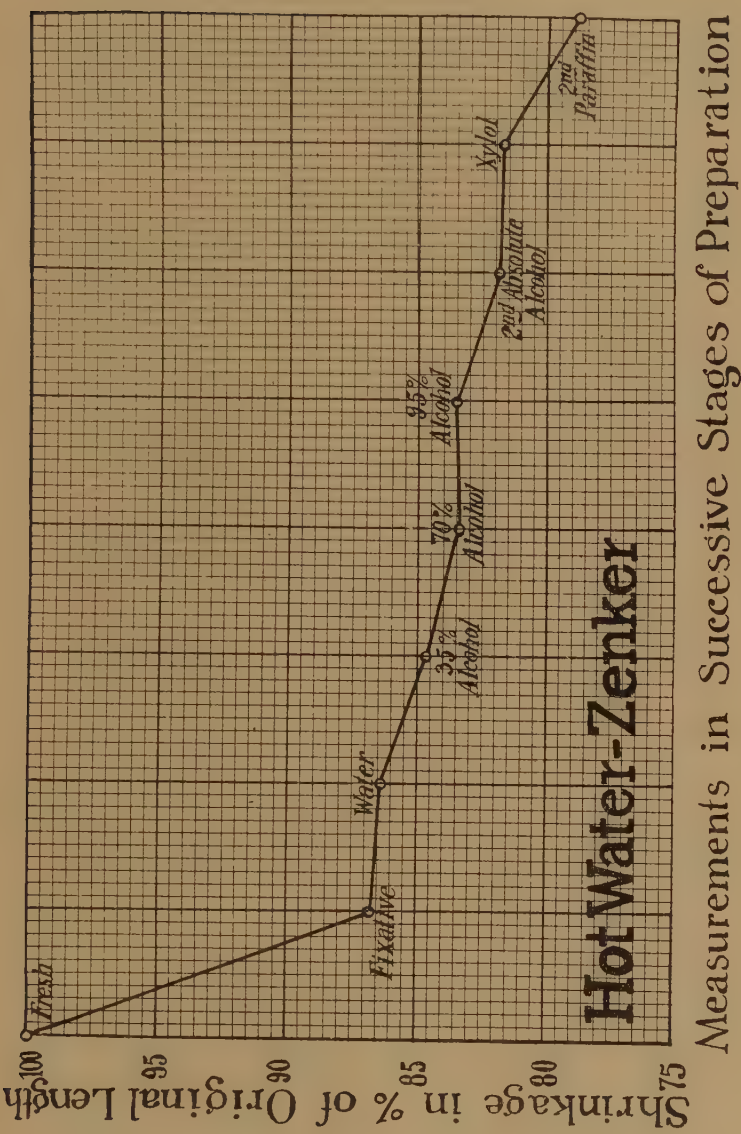


Fig. 3

Fig. 2 Curve showing the extent to which the total shrinkage of embryos during preparation for sectioning varies with the size of the embryo. Each point is located from the measurements of a single embryo.

Fig. 3 Graph showing the shrinkage induced in pig embryos by the various solutions to which they were subjected in the Zenker method modified by treatment for five minutes with water at 65°C. just before immersion in Zenker. The curve is based on the average measurements of fifteen embryos having an average original length of 18.6 mm.

The slightly less extensive shrinkage shown by the embryos treated with hot water before fixation, if significant at all, is attributable rather to the fact that their average length (18.6 mm.) was somewhat greater than the average length (16.1 mm.) of the embryos treated by Zenker alone.

Orth's fluid (formula as given in Lee, '13)

The technique employed with embryos fixed in Orth's fluid and the times the embryos were allowed to remain in the different solutions were essentially the same as that described for Zenker's solution except for the omission of the iodine treatment. The results of the measurements were tabulated as for the embryos fixed in Zenker, but since the measurements were of the same character as those given in table 1 they have not been given in detail. Figure 4 summarizes the results graphically.

The presence of formalin in Orth's fluid might be expected to lessen the shrinkage to a certain extent. Such is apparently the case, for the shrinkage in this fixative is noticeably less extensive than that in Zenker (compare fig. 4 with figs. 1 and 3). When, however, we follow out the curve, we find that the advantage is only temporary. There is a greater shrinkage in dehydration following fixation by Orth than in dehydration following Zenker fixation. The total shrinkage encountered in the two techniques is virtually identical. The transitory nature of the swelling effect of formalin fixation when it is followed by dehydration is shown much more strikingly by the data given below for formalin uncombined with other solutions.

Tellyesnicky's fluid (formula as given in Minot, '11)

The technique following fixation in Tellyesnicky's fluid was similar to that used for Zenker and Orth material. The results of the measurements are summarized in the curve of figure 5. The slightly smaller amount of shrinkage indicated on the Tellyesnicky curve as compared with the other bichromate fixatives is not sufficient to be regarded as significant. Both as regards the total amount of shrinkage induced and the stage of the process

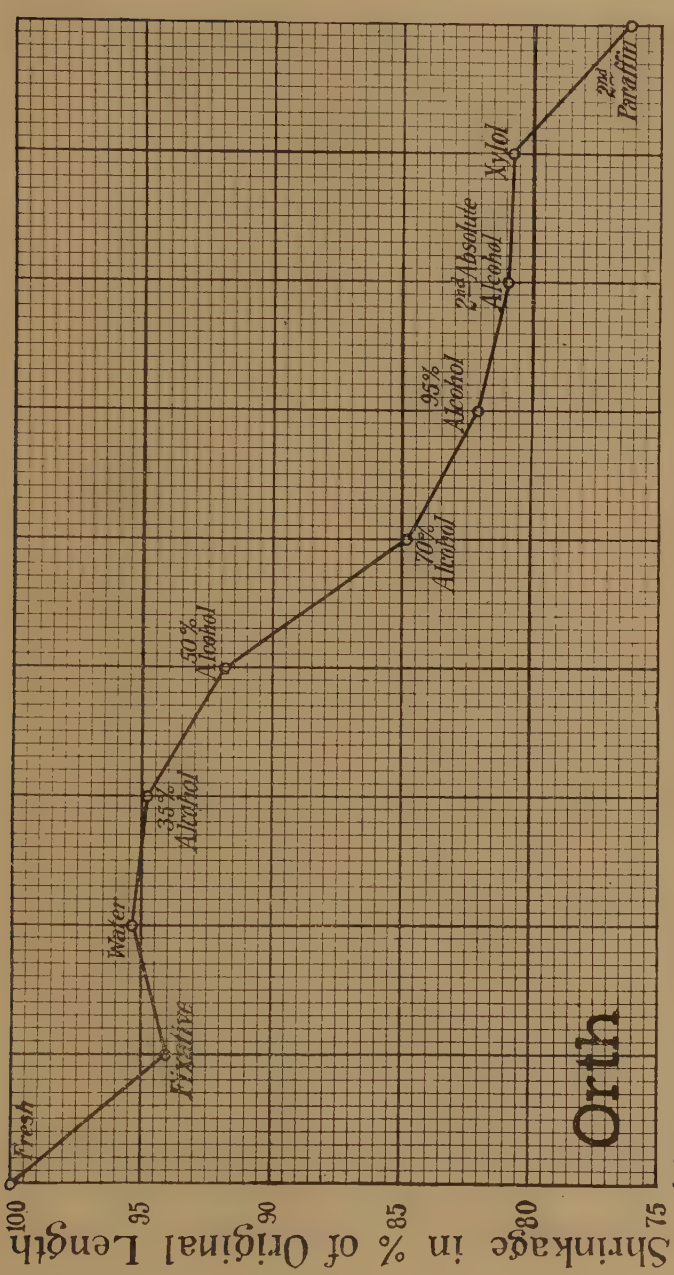


Fig. 4 Graph showing the shrinkage induced in pig embryos following fixation in Orth's fluid. The points on the curve were located from the average measurements of twenty-one embryos averaging 19.7 mm., original length.

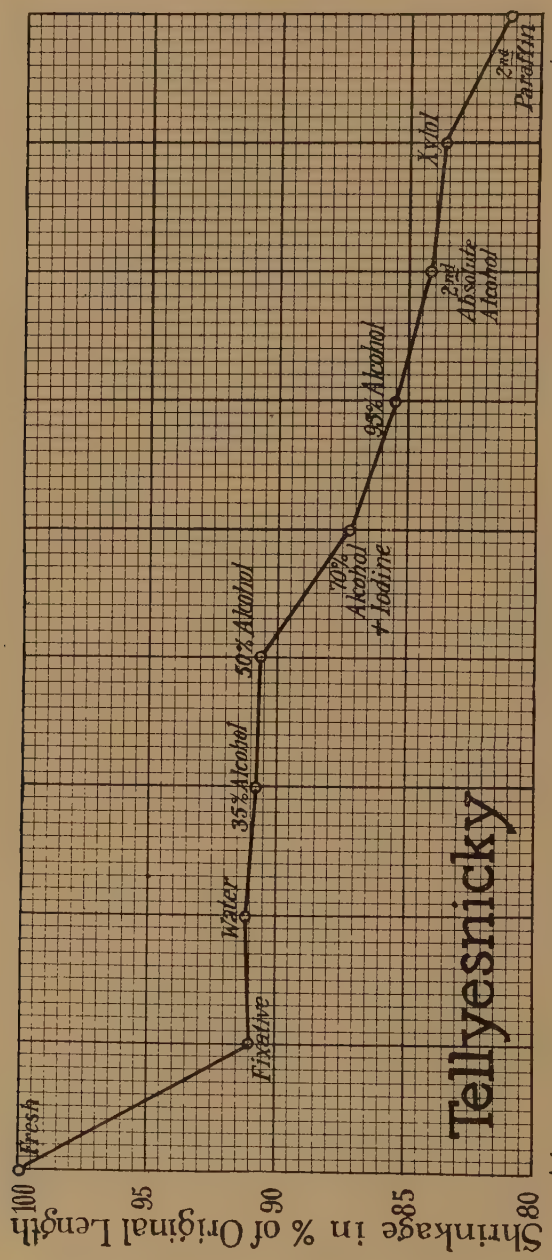


Fig. 5 Graph showing the shrinkage induced in pig embryos following fixation in Tellyesnicky's fluid. The points on the curve were located from the average measurements of twenty-four embryos, averaging 18.3 mm., original length.

at which the shrinkage takes place, Tellyesnicky, Orth, and Zenker material behave essentially alike.[2]

Formalin (10 per cent commercial formalin)

Embryos were fixed in formalin for twenty-four hours, transferred to 70 per cent alcohol overnight, left in 95 per cent alcohol twenty-four hours, in absolute (two changes) four hours, in xylol three hours, in soft paraffin two to three hours, and in hard paraffin two to three hours, according to their size.

The measurements of the embryos at various stages in the process are given in detail in table 2 and summarized in the graph of figure 6. The most striking thing that the measurements for formalin fixed embryos bring out is the fact that the initial swelling induced by the formalin is not only rapidly lost on treatment with alcohols, but gives way to marked shrinkage. In fact, the shrinkage of formalin-fixed material during dehydration is more extreme than the shrinkage encountered in dehydration following the other fixatives we used. While, therefore, formalin fixation causes an initial swelling, the ensuing processes of dehydration and infiltration result in a final shrinkage which is approximately the same as that in the bichromate processes.

Schultz ('19) found for foetuses, as did Hrdlička ('06) and King ('13) for brain material, that the initial swelling effect of formalin fixation tended to become less marked with continued storage in formalin. Although long periods in formalin reduced the initial swelling, it still left their material increased in weight as compared with its fresh condition, producing nothing approaching the shrinkage we found to be caused by dehydration of formalin-fixed embryos.

In regard to dimensions, Schultz found considerable variability, some measurements showing a slight decrease and others

[2] For adult brain material Donaldson ('94), King ('10), and Plant ('18) found an increase in weight and volume following treatment with bichromate solutions. This is in marked contrast to our findings for embryos. The difference between adult brain tissue with its compactness and high myelin content and young embryos with their loosely organized structures might possibly account for their divergent reaction in fixatives.

TABLE 2

Measurements of pig embryos, showing the amount of shrinkage which occurs in the various solutions used in the formalin (10 per cent) method of preparing material for sectioning

IDENTIFICATION NUMBER OF EMBRYO	C.-R. LENGTH IN AMNIOTIC FLUID	10 PER CENT FORMALIN		70 PER CENT ALCOHOL		95 PER CENT ALCOHOL		SECOND ABSOLUTE ALCOHOL		XYLOL		SECOND PARAFFIN	
		C.-R. length	Shrinkage	C.-R. length	Shrinkage	C.-R. length	Shrinkage	C.-R. length	Shrinkage	C.-R. length	Shrinkage	C.-R. length	Shrinkage
	mm.	*mm.*	*per cent*	*mm.*	*per cent*	*mm.*	*per cent*	*mm.*	*per cent*	*mm.*	*per cent*	*mm.*	*per cent*
F. c. 1	11.8	12.0	+1.7	10.8	8.5	10.1	14.4	9.8	16.9	9.8	16.9	9.0	23.7
F. a. 13	12.4	13.3	+7.0	11.9	3.5	10.6	14.5	10.4	16.1	10.1	18.1	9.9	20.2
F. a. 15	12.7	13.5	+5.5	11.1	12.6	10.9	14.2	10.9	14.2	10.5	17.3	10.0	21.3
F. a. 10	13.0	13.6	+4.6	11.1	14.6	11.0	15.4	10.7	17.7	10.3	20.8	10.0	23.1
F. a. 5	13.0	14.0	+7.7	11.1	14.6	10.8	16.9	10.6	18.5	10.2	21.6	9.9	23.8
F. a. 8	13.8	14.4	+4.4	11.4	17.4	11.0	20.3	11.0	20.3	10.8	21.7	10.5	23.9
F. a. 3	14.6	15.8	+8.2	12.6	13.7	12.0	17.8	12.0	17.8	11.8	19.2	11.3	22.6
F. a. 2	14.7	15.4	+4.8	12.3	16.3	11.9	19.0	11.8	20.1	11.5	21.7	11.1	24.5
F. a. 9	14.7	16.5	+12.2	13.3	9.5	13.0	11.6	12.8	12.9	12.6	14.3	11.8	19.7
F. a. 6	14.9	15.7	+8.1	12.5	16.1	12.2	18.2	12.2	18.2	12.0	19.6	11.3	24.1
F. a. 7	14.9	15.6	+4.7	12.7	14.8	12.1	18.8	12.1	18.8	11.9	20.1	11.5	22.8
F. a. 12	15.8	17.1	+8.2	14.0	11.4	13.5	14.6	13.5	14.6	13.3	15.8	12.8	19.0
F. a. 4	19.0	20.1	+5.8	16.9	11.0	16.0	15.8	15.8	16.8	15.7	17.4	14.9	21.6
F. a. 14	19.1	20.0	+4.8	17.0	11.1	16.3	14.7	16.0	16.3	15.7	17.8	15.1	20.9
F. a. 1	19.5	21.0	+7.7	17.3	11.3	17.0	11.8	16.6	14.9	16.4	16.9	15.5	20.5
F. a. 11	20.0	20.5	+2.5	17.4	13.0	16.5	17.5	16.4	18.0	16.3	18.5	15.3	23.5
F. c. 4	21.8	22.2	+1.8	20.2	7.3	18.2	16.4	18.0	17.3	17.9	17.8	16.5	24.1
F. b. 3*	23.3	24.2	+6.2	22.7	1.8	21.0	10.0	20.2	12.3	19.8	13.9	18.5	18.2
F. c. 2*	24.4	24.9	+2.0	22.7	6.8	20.8	10.8	20.6	11.8	20.0	15.4	17.5	21.1
F. c. 7	24.9	25.1	+0.8	25.1	+0.8	24.8	0.4	24.6	1.2	24.3	2.4	23.1	7.2
F. c. 5	25.1	25.3	+0.8	24.8	1.2	24.6	2.0	24.1	4.0	24.0	4.4	23.0	8.4
F. c. 6	25.8	25.9	+0.4	25.1	2.7	24.8	3.9	24.7	4.3	24.4	5.5	23.4	9.3
Average shrinkage.....		Swelling of 4.8 per cent		Shrinkage of 10 per cent		13.8 per cent		15.9 per cent		16.4 per cent		20.6 per cent	

* All the embryos in this series were measured both for crown-rump length and belly thickness. For the most part the per cent of shrinkage for the two methods of measurement corresponded very closely. In the case of the embryos indicated in the table with an asterisk these two measurements showed unusual divergence. For this reason the shrinkage figures given for them are the mean of the crown-rump and belly shrinkage. All other values are for crown-rump measurements.

a slight increase after storage in formalin. The great difference in the age of the embryos he worked on, as compared with those we dealt with, makes it inadvisable to attempt a direct comparison of our results.

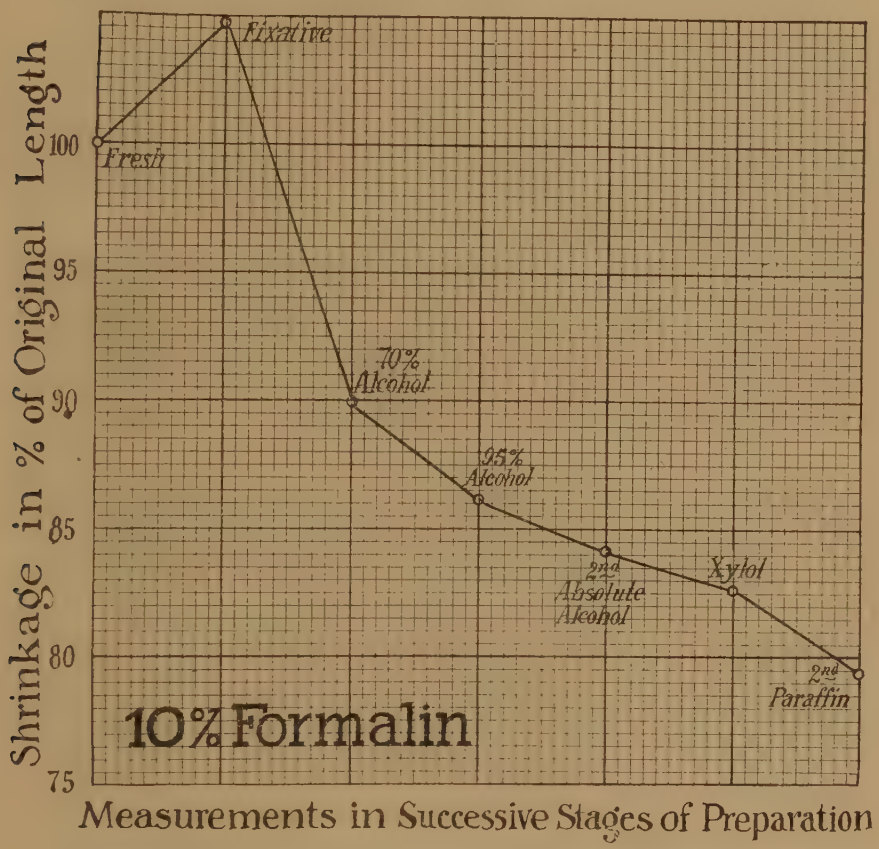


Fig. 6 Graph showing the shrinkage induced in pig embryos following fixation in 10 per cent formalin. The points on the curve were located from the average measurements of twenty-two embryos, averaging 17.7 mm., original length. Individual measurements for the same group of embryos are given in table 2.

Formol-alcohol

There are various mixtures of formalin and alcohol in use as fixatives. The formula we used was:

Formalin, (commercial)	100 cc.
Alcohol, 95 per cent	450 cc.
Acetic acid (glacial)	20 cc.
Distilled water	430 cc.

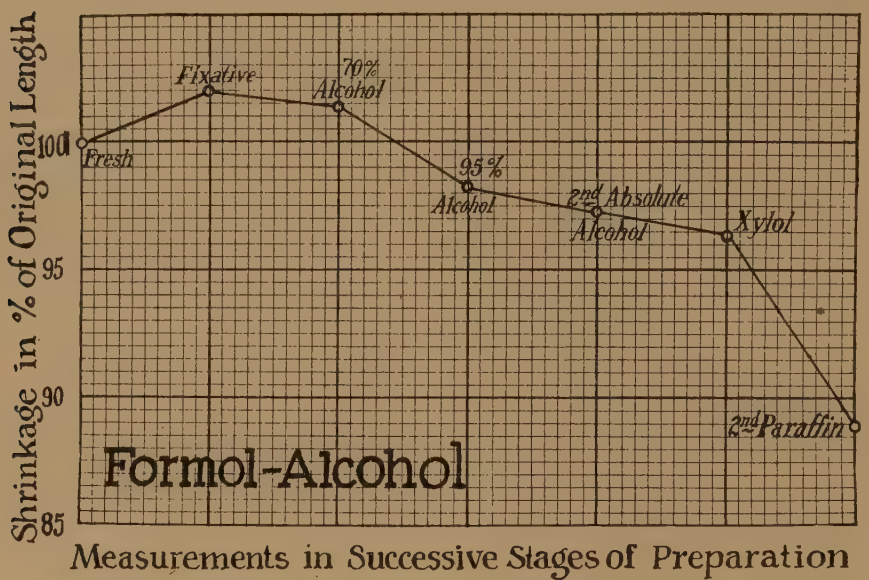


Fig. 7 Graph showing the shrinkage induced in pig embryos following fixation in formol-alcohol. The points on the curve were located from the average measurements of twenty-six embryos, averaging 19.8 mm., original length.

The technique used following formol-alcohol was similar to that given above for 10 per cent formalin. The results of the measurements are summarized in the curve of figure 7.

In embryos of the age range we worked with there was a slight initial swelling produced. This swelling was by no means as marked as that in 10 per cent formalin and could probably be altogether done away with by using more alcohol in the fixative.

The curve of figure 7 would seem to indicate that preservation of gross material with virtually no distortion from its fresh

measurements could be secured by storing material in alcohol of about 80 per cent. However, further work on this point is necessary, because, as the results of Schultz referred to above clearly show, long-continued storage in a preservative may result in unexpected changes in dimensions.

As a fixative for material to be used for histological purposes, little can be claimed for formol-alcohol. While the distorting effect of the fixative itself could undoubtedly be nullified for embryological material of a given stage as was done by Parker and Floyd ('95) for nervous tissues, there would still remain the very considerable shrinkage in dehydration and infiltration. It is true that the total shrinkage in the formol-alcohol technique, as indicated by the graph of figure 7, is somewhat less than in the bichromate fixatives, but the slight advantage on that score is more than counterbalanced by its marked inferiority as a preservative of cytological detail.

Bouin's fluid (formula as in Lee, '13)

Embryos were fixed in Bouin fifteen to eighteen hours, transferred to 70 per cent alcohol for four hours, and then to 95 per cent alcohol for twenty-four hours for hardening. After hardening they were run back through the alcohols and washed in running water overnight, and then run up to absolute, being left four hours in each of the ascending alcohols. The removal of the picric acid by this method instead of by 70 per cent alcohol was done as a matter of economy of alcohol. Except where a large quantity of material is being handled, it is without advantage. With the preliminary hardening in 95 per cent alcohol it appeared to have no deleterious effect on the fixation.

The measurements for Bouin material are summarized in figure 8. The shrinkage in the fixative is very slight (average 2.5 per cent) and in the remainder of the process is gradual and not excessive. The total shrinkage of embryos in the Bouin technique is considerably less than that encountered in the more usually employed bichromate fixatives. The preservation of cytological detail by Bouin is in no way inferior to that secured

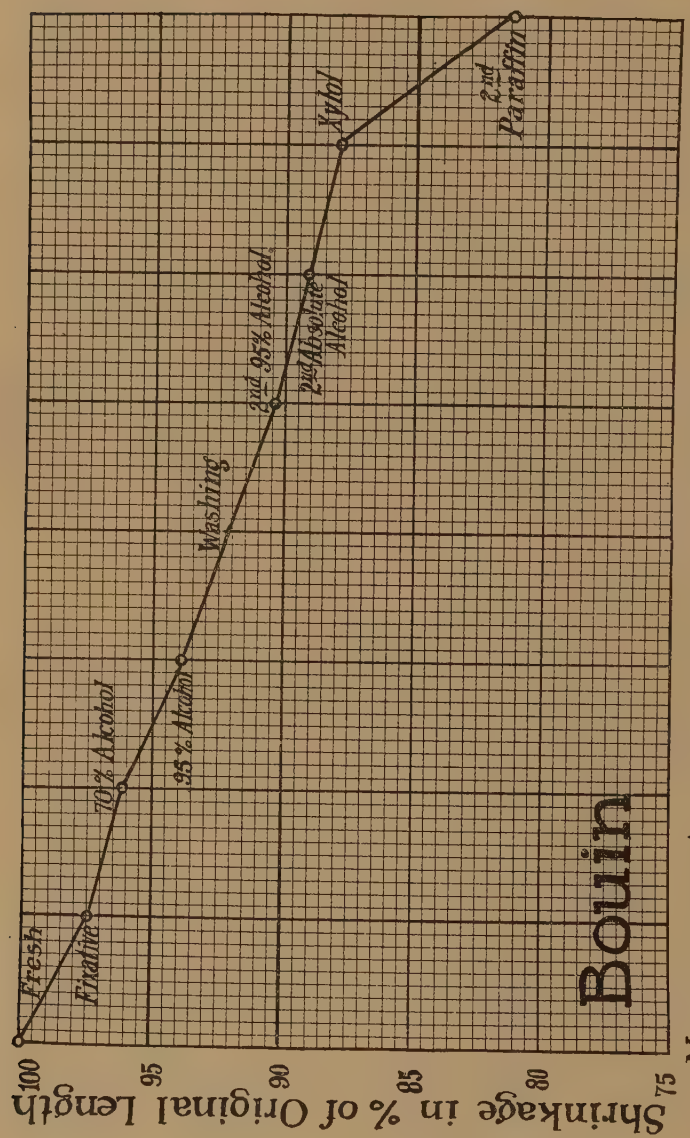


Fig. 8 Graph showing the shrinkage induced in pig embryos following fixation in Bouin's fluid. The points on the curve were located from the average measurements of twenty-three embryos, averaging 19.8 mm., original length.

by Zenker, Orth, or Tellyesnicky, and greatly superior to that secured by formalin or formol-alcohol. As to favorability for staining, Bouin material, if thoroughly washed, leaves little to be desired.

SUMMARY AND CONCLUSIONS

To ascertain the amount of shrinkage which is induced by preserving embryological material and preparing it for sectioning, series of measurements were made on pig embryos covering each stage of some of the common techniques.

The fixatives used were: Zenker, Orth, Tellyesnicky, 10 per cent formalin, formol-alcohol, and Bouin. The crown-rump length of the embryos was measured first in the amniotic fluid, and thereafter following each solution with which they were treated. The average shrinkage was computed in per cent of the original length of the embryo. From these averages graphs were plotted to show the shrinkage encountered at each step of each of the techniques used.

The shrinkage in the bichromate fixatives, Zenker, Orth, and Tellyesnicky, was the greatest encountered, the decrease in crown-rump length ranging from about 30 per cent in 10-mm. embryos to about 20 per cent in 20- to 25-mm. embryos. The more extensive shrinkage in the younger embryos is probably attributable to the less compact condition of their mesoderm. The greatest part of the shrinkage in these techniques came in the fixatives themselves.

Fixation in 10 per cent formalin resulted in an average increase in crown-rump length of about 5 per cent, which was, however, followed by rapid shrinkage during dehydration and infiltration. The total shrinkage of formalin material by the time it was embedded in paraffin was scarcely less than that for material fixed in the bichromate solutions. Histologically, the formalin-fixed material was distinctly inferior.

Formol-alcohol fixation resulted in a slight initial swelling, which was followed by marked shrinkage in dehydration and infiltration. The total shrinkage was somewhat less than that encountered in the bichromate fixatives, but the histological condition of the material was not as good.

Embryos treated with Bouin's fluid showed very slight shrinkage in the fixative. A gradual shrinkage continued, however, during dehydration and infiltration. The total shrinkage in the process was somewhat less than that following the bichromate solutions, and the histological condition of the material and its stainability were fully as satisfactory.

Comparison of the graphs for the several techniques shows:

1. There is a general tendency for the shrinkage in dehydration to be greater in the processes where the shrinkage in the fixative is less. This practically nullifies, as far as material for sectioning is concerned, the supposed advantages of a mixture such as formol-alcohol which is compounded to avoid shrinkage in the fixing fluid itself.

2. There is an abrupt increase in shrinkage during paraffin infiltration which is about the same for all the techniques used. This shrinkage encountered in molten paraffin would in all probability not occur in celloidin embedding, and would be largely reduced by the use of the celloidin-paraffin or chloroform-paraffin method of infiltration.

The crown-rump length of subsequently prepared sagittal sections showed no change from the crown-rump length of the embryo from which they were cut, as measured after paraffin infiltration.

LITERATURE CITED

DONALDSON, H. H. 1894 Preliminary observations on some changes caused in the nervous tissues by reagents commonly employed to harden them. Jour. Morph., vol. 9, pp. 123–166.

HRDLICKA, A. 1906 Brains and brain preservatives. Proc. U. S. Nat. Museum, vol. 30.

KEIBEL AND MALL 1910–12 Manual of human embryology. Lippincott, Philadelphia and London.

KING, H. D. 1910 The effect of various fixatives on the brain of the albino rat, with an account of a method of preparing this material for a study of the cells in the cortex. Anat. Rec., vol. 4, pp. 213–244.

1913 The effects of formaldehyde on the brain of the albino rat. Jour. Comp. Neur., vol. 23, pp. 283–314.

LEE, A. B. 1913 The microtomist's vade-mecum. Blakiston's Son, Philadelphia.

MINOT, C. S. 1903 Laboratory text book of embryology. Blakiston's Son, Philadelphia. 2nd ed. 1911.

PARKER, G. H., AND FLOYD, R. 1895 The preservation of mammalian brains by means of formol and alcohol. Anat. Anz., Bd. 11, S. 156–158.

PLANT, J. S. 1919 Factors influencing the behavior of the brain of the albino rat in Müller's fluid. Jour. Comp. Neur., vol. 30, pp. 411–422.

Resumen por el autor, Charles H. Miller.
Carnegie Institution of Washington.

Demonstración del esqueleto cartilaginoso en los fetos de los mamíferos.

Se pueden obtener demonstraciones muy satisfactorias del esqueleto cartilaginoso de los fetos de los mamíferos (hombre, cerdo y mono), de 20 a 150 mm. de longitud, conservados en formol o alcohol, si se tiñen con azul de toluidina, decolorando con alcohol ácido y aclarando con hidrato potásico, pudiéndose conservar de un modo permanente en glicerina los ejemplares así tratados. Ejemplares conservados a la luz durante dos años no presentan indicación alguna de decoloración.

Translation by José F. Nonidez
Cornell Medical College, New York

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, FEBRUARY 7

DEMONSTRATION OF THE CARTILAGINOUS SKELETON IN MAMMALIAN FETUSES

CHARLES H. MILLER

Department of Embryology, Carnegie Institution of Washington

In 1902 van Wijhe showed that the cartilaginous skeleton could be demonstrated perfectly in small embryos by staining the specimen with anilin dyes, especially methylene blue, then rendering it transparent in xylol and mounting in Canada balsam. His success was based on the fact that cartilage takes the stain intensely and retains it after the color has been extracted by acid alcohol from all the other tissues. Before the publication of this method it had been necessary, in order to study the morphology of the cartilaginous skeleton, to prepare the embryo in sections and model the skeleton by one of the reconstruction methods. The van Wijhe coloring method possesses a great advantage in that the entire skeleton can be prepared quickly and studied directly, with all parts in their natural connection and the contours of the whole embryo and of the different organs clearly recognizable. On the other hand, the larger specimens, which cannot be mounted in balsam and have to be preserved in xylol, soon lose their transparency and become opalescent.

Two years after the publication of the van Wijhe method, Lundvall introduced some improvements in it that served to make it more adaptable to medium-sized and large objects. He stained the cartilage with toluidin blue in place of methylene blue, and instead of xylol and balsam to render the specimens transparent, he used benzol, from which they were finally transferred to carbon bisulphide. In this fluid even large specimens will remain transparent and can be easily studied.

Both cartilage and bone can be demonstrated by the well-known potassium hydroxide clearing method of Schultze, which method has been used and experimented with in investigations

on the development of the skeletal and vascular systems by Mall, Bardeen, and Hill. Bardeen found that bone and cartilage could be made to stand out prominently by staining the embryo in alum cochineal and subsequently clearing it in potassium hydroxide. Further experience, however, has shown that such preparations are not as clear as specimens successfully prepared by the van Wijhe and Lundvall technique.

After a considerable amount of experimenting, I have found that very satisfactory results can be obtained by combining the use of the stain advocated by Lundvall with the potassium hydroxide clearing method of Schultze. In this way I have succeeded in making brilliant permanent demonstrations of the cartilaginous skeleton in pig, monkey, and human fetuses varying from 20 to 150 mm. (crown-rump length). In view of the fact that the procedure is simple and inexpensive and that it produces uniformly good results, it is to be strongly commended, and an outline of my experience with it may prove of interest to other workers.

FIXATION

Formalin. Most of the human fetuses with which I have experimented had been fixed in 10 per cent formalin solution. Among them were specimens which had been a long time in the solution and whose original condition was poor, with more or less maceration, as well as freshly fixed and well-preserved specimens. Although in the latter the staining reaction to toluidin blue is a little better, the old material nevertheless gave very satisfactory results. With formalin material other stains (alum cochineal, methylene blue, and hematoxylin) yielded poor results. Resorcin fuchsin (Weigert's) gave fair results, but much inferior to toluidin blue, which were uniformly good.

Alcohol. Fresh material fixed for six to ten days in 95 per cent alcohol, so that the embryo was well shriveled, gave excellent results with toluidin blue, as did also specimens that had been preserved for a long time in alcohol.

Bichromate acetic mixture (Müller's fluid to which 10 per cent glacial acetic acid had been added). Fresh specimens up to

40 mm. long fixed in this fluid, or material that had been fixed in it and preserved in 80 per cent alcohol for a considerable time, gave brilliant results with toluidin blue, fair results with methylene blue, and poor results with hematoxylin and with resorcin fuchsin.

STAINING

My best results in all of the various fixatives are unquestionably obtained by the use of Grübler's toluidin blue prepared according to the formula of Lundvall. Smaller embryos are thoroughly stained with it in three days; fetuses 40 mm. long should be left in the stain at room temperature for seven days, which time can be shortened, however, by the thermostat; fetuses 150 mm. long it is necessary to leave in the stain at least ten days. Before staining it is well to take the precaution of neutralizing any residual acid from the formalin by immersing in ammonia water overnight. After staining, the specimen is decolorized in acid alcohol (1 per cent hydrochloric acid in 70 per cent alcohol), which, for small specimens, requires about seven days and for large specimens ten days. The acid alcohol is changed daily until the fluid is only slightly tinged with the extracted stain, and the specimen is then ready for clearing.

Next to toluidin blue, methylene blue gave the most satisfactory results. The disadvantage of this stain, however, is that there is some danger of carrying the decolorization too far. The color is extracted more rapidly by the acid alcohol. Small embryos should not be left in the decolorizing fluid more than two days. A further disadvantage is that the color of the specimen subsequently fades much more rapidly than after toluidin blue. Formalin specimens, stained in resorcin fuchsin, gave fair results. This stain is more resistant to acid alcohol than either methylene blue or toluidin blue, and it is more difficult to thoroughly extract the color from the other tissues; hence the specimens are not so brilliant. Both hematoxylin and alum cochineal were followed by poor results, owing to the difficulty in securing uniform decolorization and to the fact that, in clearing the specimen in potassium hydroxide, the remaining stain was completely removed.

CLEARING

Small embryos can be easily rendered transparent by passing them through the graded alcohols, then benzol, and finally into oil of wintergreen, after the Spalteholz method, or into carbon bisulphide, according to Lundvall. With larger fetuses (over 110 mm. long) we have experienced some difficulty in making them completely transparent. On account of this, resort was had to potassium hydroxide, which usually cleared even the largest specimens and without any detriment to the stain. In one instance (a human fetus 258 mm. long), although the greater part of the specimen cleared satisfactorily, there remained some irregular milky patches which would not clear. Resort was had to absolute alcohol and ether, on the basis that the failure was due to the subcutaneous fat. This treatment, however, did not remove the difficulty, which is still unexplained.

Specimens may be transferred directly from the acid alcohol to 2 per cent potassium hydroxide, but this tends to soften the tissue. It was therefore found better to thoroughly harden the specimen in 95 per cent alcohol before clearing. Embryos 20 mm. long will clear in 2 per cent potassium hydroxide in two days at an average room temperature, after which they should be transferred to 20 per cent glycerin. For fetuses 40 mm. long four days is sufficient. The rapidity with which the specimens become clear is influenced greatly by the temperature; in summer the process is much more rapid than in winter. With large fetuses it is well to start with 2 per cent potassium hydroxide and after two changes to increase the strength to 3 per cent. They should stay in the latter solution until they are transparent, which may require seven days or longer. On transferring to glycerin, if it is found that the specimen is not completely transparent, it can be safely returned to 3 per cent potassium hydroxide for further clearing. After the specimen is cleared it is passed through graded glycerin, beginning with 20 per cent and increasing 20 per cent every two days, until it is finally stored in pure glycerin, to which has been added a crystal of thymol to prevent mold. The clearing of larger specimens is facilitated by removing the brain through the anterior fontanelle and the

viscera through a median incision in the abdomen. In the permanent storing of such material it is well to take the precaution of protecting it from the light. I have some specimens that have been kept in subdued light for two years and which show scarcely any indication of fading.

SUMMARY

The complete method that we are now using in this laboratory for preparing specimens for the demonstration of the cartilaginous skeleton may be summarized as follows:

1. Formalin material is first washed overnight in water to which a few drops of ammonia have been added.

2. Transferred to 70 per cent alcohol, where it is left for from seven to fourteen days, changing the alcohol daily for the first five days.

3. Stained in Lundvall's solution of toluidin blue (Grübler's toluidin blue, 1 gram; alcohol 70 per cent, 400 cc.; HCl, 4 cc.).

4. Decolorized in 70 per cent alcohol, 100 cc.; HCl, 1 cc., for from seven to ten days, until the alcohol is but slightly tinged with the stain.

5. Transferred to 80 per cent alcohol, then to 95 per cent alcohol, three days in each.

6. Transferred to 2 per cent KOH in distilled water, where it is left until cleared, usually two or three days.

7. Transferred to glycerin, 20 per cent, 40 per cent, 60 per cent, and 80 per cent, respectively, in distilled water, two days or more in each; the larger the specimen, the longer it should remain in each grade of glycerin.

8. Stored in pure glycerin to which have been added a few crystals of thymol to prevent mold.

LITERATURE CITED

LUNDVALL, H. 1904 Ueber Demonstration embryonaler Knorpelskelette. Anat. Anz., Bd. 25, S. 219–223.

SCHULTZE, O. 1897 Ueber Herstellung und Conservierung durchsichtiger Embryonen zum Studium der Skeletbildung. Anat. Anz., Bd. 13 (Verhandl. der Anat. Gesellsch.), S. 3–5.

VAN WIJHE, J. 1902 A new method of demonstrating cartilaginous microskeletons. Proc., Koninklijke Akademie van Wetenschappen te Amsterdam, May 31.

Resumen por el autor, Alexander Gibson.

Nota sobre la persistencia del conducto de Cuvier izquierdo.

El ejemplar objeto de la presente nota fué hallado en un varón de 60 años de edad. Ilustra de modo muy claro la obliteración incompleta del conducto de Cuvier izquierdo; en otras palabras, existe una doble vena cava superior, si bien su calibre es mucho menor que el de la vena cava superior derecha. Esta condición ha sido mencionada unas sesenta veces, pero no se han obtenido datos precisos sobre la frecuencia de su ocurrencia. La explicación de esta anomalía es sencilla si se tiene en cuenta el carácter de los canales venosos que entran en el corazón. Las venas cardinal anterior y posterior se unen a cada lado del cuerpo para formar los conductos de Cuvier. Estos últimos vierten la sangre en el seno venoso del corazón, el conducto izquierdo en el asta izquierda del seno, el derecho en el asta derecha. El asta izquierda del seno no se desarrolla y a consecuencia de esto el conducto de Cuvier izquierdo se atrofia.

Normalmente los únicos restos presentes son la vena oblícua del corazón y el ligamento de la vena cava izquierda, el cual se insinúa entre la vena pulmonar superior izquierda y la superficie inferior de la arteria pulmonar. La vena intercostal superior izquierda representa las porciones terminales de las venas cardinales anterior y posterior. En el sujeto descrito se observa la existencia de un canal venoso permeable que pasa desde la vena intercostal superior izquierda hasta el extremo inferior izquierdo del seno coronario.

Translation by José F. Nonidez
Cornell Medical College, New York

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, FEBRUARY 28

NOTE ON A PERSISTENT LEFT DUCT OF CUVIER

ALEXANDER GIBSON
University of Manitoba

ONE FIGURE

The specimen illustrated was obtained from a male subject aged sixty. It illustrates very clearly incomplete obliteration of the left duct of Cuvier; in other words, a double superior vena cava is present, although the calibre of the channel is much less than is that of the right superior vena cava.

The condition has been reported some sixty times. No facts as to the frequency of its occurrence have been obtained. McCotter states that he has observed it once only among seven hundred dissecting-room subjects.

The explanation of the condition is a simple one if reference be made to the venous blood channels entering the heart. On both sides of the body the anterior cardinal vein and posterior cardinal vein unite to form the duct of Cuvier. The duct of Cuvier pours its blood into the sinus portion of the heart, the left duct into the left horn of the sinus, the right duct into the right horn. The left horn of the sinus fails to develop, and coincidently the left duct of cuvier atrophies. Normally, the only remnants present are the oblique vein of the heart, and the ligamentum venae cavae sinistrae, which latter stretches between upper left pulmonary vein and under surface of pulmonary artery. The left superior intercostal vein is the representative of the terminal portions of anterior and posterior cardinal veins.

In this specimen a previous venous channel is seen passing from the left superior intercostal vein down to the left extremity of the coronary sinus. It will be observed to arise from the left superior intercostal vein at a well-marked angle between horizontal and vertical portions of the vein.

BIBLIOGRAPHY

Antonelli 1882 Res della R. Academia med-chir di Napoli.
Boyd Journal Anatomy and Physiology v. 28.
Cameron Journal Anatomy and Physiology, v. 49, p. 140.
McCotter 1915 Journ. Mich. Med. Soc.
Weigert 1881 Virchow's Archow's Archiv.
Le Blanc Guy's Hospital Gazette, v. 29.

Figure 1

COUNTWAY LIBRARY
HC 6906